Improving rabies control in free-roaming dogs

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Abstract

Canine-mediated rabies is a serious zoonosis responsible for at least 55,000 human deaths every year, primarily in less developed communities in Asia and Africa where domestic dogs are free-roaming. The disease can be effectively controlled through vaccinating at least 45% of the dogs in a population; however the impact of vaccinations on disease incidence may be affected by dynamic demographic and immunological processes. Specifically, the contribution of these processes, and their regulatory factors, to vaccination coverage and rabies transmission has not been comprehensively estimated. To improve rabies control, through field interventions and epidemiological modelling, more information regarding the effect of these processes, and their regulatory factors, on population and disease dynamics and vaccinal responses was needed. This required a multifaceted approach, using techniques from the fields of population ecology, vaccine-immunology, social science and epidemiological modelling. Demographic data were collected from four populations of free-roaming domestic dogs, two in South Africa and two in Indonesia where rabies is endemic. Longitudinal, individual-level data were obtained by direct observation and surveys, and community-level data by participatory methods. Longitudinal, serological data were collected from three cohorts within the populations. Epidemiological models were based on epidemic theory and empirical data from this current study and previous studies.

A wide array of data were generated relevant to planning rabies control programmes, however of particular importance was evidence regarding positive and negative the impacts of human factors on population and disease dynamics. Nearly all of the dogs were owned, despite being free-roaming, and were accessible for vaccination through their owners; and population size was regulated through human demand for dogs and a substantial fraction of dogs was acquired from outside the communities. These translocated dogs may contribute to the spread of rabies, necessitating widespread and sustained vaccination programmes. Considerable differences in the handleability of dogs between locations and, thus ease of vaccine delivery, may also be attributable to differences in human-dog interactions. Finally, a critical review of the literature, and evaluation of epidemiological models, suggests that human interference in the transmission processes may reduce the incidence of rabies and vaccination threshold.

This study has provided specific evidence that human behaviours are likely to be critically important in relation to the transmission and control of canine-mediated rabies – and is the first to properly identify this. Further detailed studies are required to explore these behaviours

and how they vary culturally and geographically. In addition, the results highlight the critical role that demographic processes more generally, as well as immunological decay, play in influencing the long term success of rabies vaccination programmes. Overall, this research has provided valuable support for planning rabies control programmes and for parameterisation of epidemiological models of infectious diseases, including rabies.

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Table of Collaborators

Individual providing assistance	Organization	Contribution
Prof James Wood	University of Cambridge	PhD supervisor
Prof Sarah Cleaveland	University of Glasgow	PhD advisor
Dr Helen Whay	University of Bristol	PhD advisor
Prof Johan Schoeman	University of Pretoria	Collaborator for studies in South Africa
Prof I Made Damriyasa	Udayana University (Bali)	Collaborator for studies in Indonesia
Sherry Grant, Luh Putu Listriani Wistawan	Yudisthira Foundation and Indonesian Animal Welfare (InAW)	Legal entity for research permits
Data collection under MMs supervision		
CLAW employees	Community Led Animal Welfare (NGO, Johannesburg)	Translation, enumeration, dog handling
InAW employees	Indonesian Animal Welfare (NGO, Bali)	Translation, enumeration, dog handling, vaccination, facilitation of the PRA
BAWA employees	Bali Animal Welfare Association (NGO, Bali)	Assistance with facilitation of PRA
Yudisthira employees	Yudisthira Foundation (NGO, Bali)	Assistance with facilitation of PRA
Sowmyaa Bharadwaj	Praxis Institute for Participatory Practices (India)	Development of PRA exercises, team training and facilitation of PRA
Laboratory analysis		
Prof Amelia Goddard, Cheryl Booth, Gertie Pretorius, Carien Muller	University of Pretoria	Serum sample storage, complete cell counts and microscopy
Dr Volker Schwann	University of Pretoria	Faecal analysis
Prof Anthony Fooks, Dr Daniel Horton, Trudy Goddard, David Seldon, Hooman Goharriz	Weybridge Animal Health Veterinary Laboratory Agency (UK)	FAVN assays (pipetting, plate preparation and reading), LBV assays
Data analysis		
Dr TJ McKinley	University of Cambridge	Provided code for Monte Carlo estimates of early pregnancy, developed and fitted ordinal regression models (Chapter 2)
Dr Andrew Conlan	University of Cambridge	Modification of formula to estimate R_0 (Chapters 1 & 5 equations 1-3); provided the model code used in Chapter 5
Co-authors on papers (chapters)		
Prof James Wood; Dr TJ McKinley; Dr Andrew Conlan; Dr Olivier Restif; Prof Sarah Cleaveland; Dr Katie Hampson; Dr Helen Whay; Prof Anthony Fooks; Dr Daniel Horton; Prof Johan Schoeman; Prof Amelia Goddard; Prof I Made Damriyasa; Sowmyaa Bharadwaj	see above	Thoroughly reviewed the manuscripts; provided extensive technical advice and guidance specific to each manuscript [in particular OR & AC Chapter 1; TJM Chapters 2 & 4; SW Chapter 3; AC Chapter 5]

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List of Abbreviations

Abbreviation	Meaning
AIC	Akaike's information criterion
BCS	Body condition score
CBC	Complete blood count
CI	Confidence interval
CLAW	Community Led Animal Welfare
CM	Compartmental model
cm	Centimetre
DA2PPv	Canine Distemper, Adenovirus Type 1 & 2, Parainfluenza and Parvovirus
DoA	Department of Agriculture
DoL	Department of Livestock
DSS	Deep skin scrape
EDTA	Ethylene diamine-tetraacetic acid
FAVN	Fluorescent antibody virus neutralization assay
GMT	Geometric mean titre
GPS	Global positioning system
HIPD	Hunting indicator of population density
IBM	Individual based model
ICAM	International Companion Animal Management Coalition
IFAW	International Fund for Animal Welfare
InAW	Indonesia Animal Welfare
IU	International Unit
km	Kilometre
LBV	Lagos Bat Virus
ml	Millilitre
NGO	Non-governmental organization
nlme	Non-linear mixed effects
NPO	Non-proportional odds
OIE	Office International des Epizooties / World Organisation for Animal Health
OPL	Oestrus, pregnancy, lactation
PO	Proportional odds
PPA	Posterior probability of association
PRA	Participatory rural appraisal
RABV	Rabies virus
SD	Standard deviation
SE	Standard error
SEI	Susceptible-Exposed-Infectious
VNA	Virus neutralizing antibody
VP	Vaccination point
WHO	World Health Organization
WSPA	World Society for the Protection of Animals

List of Symbols

Symbol	Meaning
^{o}C	Degrees Celsius
cm^2	Area in centimetres
km^2	Area in kilometres
^{o}E	Degrees latitude
^{o}S	Degrees longitude
ln	Natural log
K	Carrying capacity
%	Percentage
p	p-value
R_0	Basic reproductive number
Chapter 4	
$\boldsymbol{\beta}$	Regression coefficient
n	Sample size
$\boldsymbol{N}$	Normal distribution
$\boldsymbol{r}$	Correlation coefficient
$\boldsymbol{X}$	Regression covariate
$\boldsymbol{Y}$	Response variable
$\boldsymbol{\theta}$	Individual-level random effect term
$\boldsymbol{\sigma^2}$	Variance
$\boldsymbol{\varepsilon}$	Error term
$\sum$	Sum
Chapter 5	
$\boldsymbol{\alpha}$	Removal rate that scales with population density
$\boldsymbol{A}$	Area
$\boldsymbol{b}$	Average gain rate
$\boldsymbol{\beta}$	Transmission rate
$\boldsymbol{\delta t}$	Time step
$\boldsymbol{\gamma}$	Removal rate
$\boldsymbol{1/\gamma}$	Infectious period
$\boldsymbol{m}$	Removal rate without human interference
$\boldsymbol{\mu}$	Average loss rate
$\boldsymbol{n}$	Population density
$\boldsymbol{N}$	Population size
$\boldsymbol{1/\sigma}$	Latent period

Preface

Canine-mediated rabies claims tens of thousands of human lives every year, primarily in low-income communities in Asia and Africa. Although culling (i.e. the widespread killing of dogs regardless of infection status) is often used as the primary means to control the disease, mass vaccination of dog populations should be the main stay of effective control (WHO 2013). Planning vaccination campaigns depends on knowledge of the size and accessibility of dog populations and of rates of decline in vaccination coverage to below protective levels. This in turn requires an understanding of the demography and immune responses to rabies vaccine of domestic dogs. Understanding the factors that drive disease transmission, facilitating development of epidemiological models of local and spatial dynamics and for movement controls, can also support effective rabies control. Demographic data are also needed to plan canine population controls that are often used to address animal welfare and other public health concerns. However, there is a paucity of demographic and immunological data in the literature necessary for rabies and population control, and apparently an incomplete understanding of the drivers of local transmission of rabies, reflected in frequent culling dogs for rabies control.

The existing literature includes various aspects of dog ecology that contribute substantially to rabies and population control. However, except for a limited number of more comprehensive studies of dog population dynamics (Boitani *et al.* 1995; Pal 2001), generally only facets of dog demography are reported in various studies of canine rabies, from a broad range of different study types. Any demographic data had generally been collected in cross-sectional studies, relying on owner recall for essential information, such as reproductive histories; most of these studies included only a sub-set or sample of households or dogs, often from large geographical areas. A further complication is that conflicting hypotheses regarding the factors that drive population and disease dynamics are presented. This may be, in part, a consequence of extrapolations of assumptions and observations of population and disease dynamics from wildlife to domestic dogs. For example, the WHO/WSPA Guidelines for Dog Population Management (WHO & WSPA 1990), frequently referred to by those implementing rabies and population controls, policy makers and academics, states that free-roaming dog population size is regulated by environmental resource constraints similar to wildlife (i.e. the dogs are effectively unowned), but also that free-roaming dogs generally have a reference household (i.e. the dogs are owned). Furthermore, serological studies of rabies vaccine-induced immune responses are generally limited to laboratory or pet dogs and

not to the free-roaming dogs that frequently transmit the disease. Finally, there is paucity in the literature regarding the factors that drive rabies transmission.

This study aimed to address these limitations by generating individual-level, demographic and serological data, necessary for rabies control, from four entire populations through longitudinal, direct observations and owner questionnaires restricted to the recent past (described in Chapters 2 and 4). Community-level participatory exercises were also used as a novel approach to assess key aspects of ownership (described in Chapter 3). A thorough review of the role of population density in disease transmission was also undertaken in the context of population density-reduction through culling for rabies control (described in Chapter 1).

My involvement in this research project began as Program Manager of the global Companion Animals Program for the International Fund for Animal Welfare (IFAW), faced with writing organizational policy on dog population management but also the aforementioned fundamental gaps and incongruities in the literature. I was also travelling extensively to observe population control measures in free-roaming dog populations and increasingly questioned the efficacy of these interventions. For similar reasons, the International Companion Animals Management Coalition (ICAM) http://www.icam-coalition.org/ was founded by Dr Elly Hiby of the World Society for the Protection of Animals (WSPA), myself (IFAW) and colleagues from several other organizations leading in dog population management. Collectively, these factors were the catalyst for my decision to undertake my PhD studies, to generate data directly relevant to my funders' programmatic work on population and rabies control. The research was undertaken in collaboration with Pretoria and Udayana Universities and the support of several researchers in the field of rabies control, disease dynamics and animal welfare based at Glasgow, Cambridge and Bristol Universities. A part-time PhD allowed me to continue to engage with my funders and ICAM directly as a conduit of current information between the field and round table. It also provided the massive benefit of a sufficient period to collect a more extensive data set than would have been possible with a full-time PhD, with real, practical advantages for rabies and population control. Likewise, the luxury of time in the field afforded an intimidate knowledge of the communities by myself and the field teams, which was fairly unique and hugely advantageous to this type of research.

The research was initially designed to evaluate demographic processes, and their regulatory factors, at the local level, for the purpose of improving population control. However, given

that most of the literature relevant dog ecology was in the context of rabies control, the broad application of these demographic data to rabies control became rapidly apparent. Similarly, the need for, and opportunity to incorporate, a serological sub-study to assess rabies vaccine-induced immune responses into the project became clear early after inception. The inclusion of a serological study was also relevant to IFAW and WSPA given their increasing involvement in canine-mediated rabies control, especially issues surrounding culling but also the implementation of vaccination programmes.

Soon after initiating the study, a rabies outbreak occurred in Bali. One of the initial responses of the provincial government was extensive culling of the free-roaming dogs in an attempt to control the disease. In collaboration with world experts in rabies control and endorsements by the ICAM members, this precipitated my writing of recommendations for the provincial government for rabies control based on mass vaccination. In turn, this initiated my detailed review of the literature regarding the role population density in rabies transmission and the efficacy of culling for disease control. The extension of the cull to an area where I was conducting the research (thankfully at the tail end of data collection) added fuel to fire with regards publishing my review on culling. It also initiated a follow-up study given the unique opportunity to monitor the impact of culling on population dynamics, particularly focusing on human behaviour, where population dynamics had been previously monitored (thus providing historic control data). This follow-up was subsequently extended to monitor the effects of population sterilization and other veterinary interventions on population dynamics and animal welfare in the other research sites.

Demographic, immunological and epidemiological data are integral to disease and population dynamics and controls. Therefore, this dissertation was deliberately written as a series of inter-related papers, with the view to publication in the hope that the information will be readily accessible to and helpful for those currently in the field implementing rabies controls, policy makers and academics.

Chapter 1 (published) examines the factors that drive the transmission of rabies, specifically host population density and the reasons why density reduction, particularly through culling, fails to control rabies. The discussion introduces the role of human factors in the transmission and control of rabies. [*M.K Morters, O Restif, K Hampson, S Cleaveland, J.L.N Wood, A.J.K Conlan. 2012. Evidence-based control of canine rabies: a critical review of population density reduction. Journal of Animal Ecology, 82, 6-14.*]

Chapter 2 (provisionally accepted 26 March, 2014) presents demographic data from four free-roaming dog populations, and includes evaluation of the regulatory effect of human behaviour on dog population dynamics and declines in rabies vaccination coverage following a pulse vaccination campaign. [*M.K Morters, T.J McKinley, O Restif, A.J.K Conlan, K Hampson, H.R Whay, I Made Damriyasa, J.L.N Wood. The demography of free-roaming dog populations and applications to disease and population control. Journal of Applied Ecology.*]

Chapter 3 (provisionally accepted 27 February, 2014) describes community-level participatory methods applied to the study of dog ownership in Bali. This is a novel approach to assess the fraction of unowned dogs in populations, which complements the ecological study described in Chapter 2. [*M.K Morters, S Bharadwaj, H.R Whay, S Cleaveland, I Made Damriyasa, J.L.N Wood. Participatory methods for the assessment of the ownership status of free-roaming dogs in Bali, Indonesia, for disease control and animal welfare. Preventive Veterinary Medicine.*]

Chapter 4 (under review) evaluates rabies vaccine-induced immune responses in free-roaming dogs, including the effects of waning immunity and demographic processes on vaccination coverage. [*M.K Morters, T.J McKinley, D.L Horton, S Cleaveland, J.P Schoeman, O Restif, H.R Whay, A Goddard, A.R Fooks, I Made Damriyasa, J.L.N Wood. Achieving population-level immunity to rabies in free-roaming dogs in Africa and Asia. PLoS Neglected Tropical Diseases.*]

Chapter 5 explores the transmission dynamics of canine rabies using models that account for human interference in transmission as proposed in Chapter 1, demographic data from Chapter 2 and previously reported epidemiological parameter estimates. This is being prepared for publication and, thus far, has received input from *A.J.K Conlan, O Restif and J.L.N Wood.*

Finally, Chapter 6 summarizes the results from the study and draws conclusions on the implications of the research and areas where further research is required.

Cross-referencing in this thesis uses chapter number. Supporting documents, tables and figures from the papers are provided in appendices. The appendices have been submitted to the journals as supporting materials to the papers listed above.

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text and in the Table of Collaborators (see page v).

This research was approved by the Ethics Committee, University of Cambridge, and the Animal Ethics Committee, University of Pretoria. Permits to collect demographic data were granted by the Ministry for Research and Technology (RISTEK), Indonesia. Blood samples were collected under the auspices of the Faculty of Veterinary Medicine, Udayana University, Bali, and permits for vaccination and blood collection were granted by the Balinese provincial and regencies Departments of Livestock, the districts Centres of Animal Health (UPT), and Kesbang, Pol and Linmas (the combined Agencies for National Unity, Politics and Protection). In all of the sites, informed consent was obtained prior to each survey and blood test from the community leaders and owners, who were kept fully informed of the purpose, approach and progress of the study. Survey respondents under 16 years of age were always interviewed with an adult present. Vaccination and blood sampling were only carried out with the owner, or responsible adult delegated by the owner, present and their express consent.

The dissertation does not exceed the required word limit. Word count: 39,819

Chapter 1

Evidence-based control of canine rabies: a critical review of population density reduction

Summary

Control measures for canine rabies include vaccination and reducing population density through culling or sterilization. Despite evidence that culling fails to control canine rabies, efforts to reduce canine population density continue in many parts of the world. The rationale for reducing population density is that rabies transmission is density-dependent, with disease incidence increasing directly with host density. This may be based, in part, on an incomplete interpretation of historical field data for wildlife, with important implications for disease control in dog populations. Here we examine historical and more recent field data, in the context of host ecology and epidemic theory, to understand better the role of density in rabies transmission and the reasons why culling fails to control rabies. We conclude that the relationship between host density, disease incidence and other factors is complex and may differ between species. This highlights the difficulties of interpreting field data and the constraints of extrapolations between species, particularly in terms of control policies. We also propose that the complex interactions between dogs and people may render culling of free-roaming dogs ineffective irrespective of the relationship between host density and disease incidence. We conclude that vaccination is the most effective means to control rabies in all species.

1.1 Introduction

Canine-mediated rabies is a serious zoonosis causing an estimated 55,000 human deaths per year (Knobel *et al.* 2005). Mortality from rabies is highest in developing communities in Africa and Asia where domestic dogs are predominately free-roaming (Ezeokoli & Umoh 1987; Butler & Bingham 2000; Kitala *et al.* 2002; Kayali *et al.* 2003a; Windiyaningsih *et al.* 2004; Kasempimolporn, Jitapunkul & Sitprija 2008). Social, economic and political factors contribute to the inadequate control of rabies in domestic dog populations (WHO 2004), accentuated by an incomplete understanding of disease dynamics. Knowledge of the factors that drive the transmission of rabies is needed for development of effective, sustainable disease control measures.

Two main methods are used to control canine rabies: vaccination (Cleaveland *et al.* 2003; WHO 2004; Schneider *et al.* 2005; Cleaveland *et al.* 2006) and measures aiming to reduce dog population density, usually by culling (i.e. the widespread killing of dogs regardless of infection status) (Beran & Frith 1988; Windiyaningsih *et al.* 2004) but also by sterilization (WHO 2004; Reece & Chawla 2006). Dog vaccinations are often undertaken as annual campaigns that aim to achieve 70% coverage (WHO 2004). This target coverage is supported by empirical evidence and theory, which indicates that a 70% coverage achieved during campaigns should maintain population immunity above the critical levels (20-45%) required to interrupt rabies transmission (Coleman & Dye 1996; Cleaveland *et al.* 2003; Hampson *et al.* 2009). This additional coverage above the critical level compensates for the loss in coverage arising from an increase in susceptible and loss of immune dogs through demographic and immunological processes (Hampson *et al.* 2009). Culling of dogs is also used, alone or with vaccination (Kaplan, Goor & Tierkel 1954; Larghi *et al.* 1988), based on the assumption that a physical reduction in the number of dogs must reduce the incidence of rabies despite evidence suggesting that it is ineffective (Beran & Frith 1988; WHO 2004; Windiyaningsih *et al.* 2004). Culling is still used, partly as a visible response to public concerns about rabies. It is also perceived to be easier to implement than annual vaccination of 70% of dogs, particularly if many are free-roaming and poorly socialized, and in areas where veterinarians and animal health workers have relatively little experience or confidence in handling dogs. In some areas, sterilizations are carried out together with vaccinations, on the basis that this is a more humane and culturally acceptable approach to reducing dog population density.

The theoretical basis for rabies control measures involving culling or sterilization is the assumption that rates of transmission are density-dependent (Anderson *et al.* 1981; Wandeler *et al.* 1988; Cleaveland 1998; Hampson *et al.* 2007). This scaling of transmission rates occurs if the rate of encounters between susceptible and infectious individuals increases with host population density. Under this assumption, we expect that disease incidence will also increase with host density, as will the basic reproductive number (R_0) that characterises the maximum reproductive potential of a pathogen. R_0 is defined as the average number of secondary infections produced when one infected individual is introduced into a wholly susceptible population (Anderson & May 1991). For an epidemic to spread, R_0 must, by definition, be >1. Hence, under density-dependent transmission there will exist a threshold density below which disease cannot invade a population. This contrasts with frequency-dependent disease transmission where the rate of contact and subsequent rates of transmission are assumed to be

independent of host density and a threshold density for invasion does not exist (Begon *et al.* 2002; Lloyd-Smith *et al.* 2005).

Under either frequency- or density-dependent transmission, vaccination equally reduces both the number and proportion of susceptible individuals in a host population, and thus, the opportunities for transmission to occur. Therefore, the assumption that rabies transmission is density-dependent has little consequence for the efficiency of vaccination programmes. Conversely, the assumption is of critical importance with regards to control measures that aim to reduce dog population density. The net impact of culling and sterilization on subsequent rates of rabies transmission depends on the degree to which transmission scales with population density. Under the assumption of frequency dependence, density reduction will have no impact on the rate of transmission. Conversely, when transmission is density-dependent, there will be a threshold for disease invasion, and density reduction alone has the potential to achieve disease eradication. However, stochastic effects and antagonistic biological processes may complicate these simple relationships.

Establishing the relationships between host density, disease incidence and other processes is therefore not only important for refinement of epidemiological models for rabies transmission, but has serious practical implications for the utility of density reduction in controlling rabies. In this study, we review current understanding of the role of density and other factors in rabies transmission in dogs in order to encourage reappraisal of the most appropriate and effective means of rabies control. Within the literature, and during the development of policy, extrapolations are often made between species, in particular between wildlife and domestic dogs. We therefore extend our review to rabies transmission in wildlife and highlight the differences and similarities with dog populations. We also compare the utility of various lines of evidence between species. This discussion will focus on fox rabies in particular, as empirical data on the local transmission of wildlife rabies are largely confined to this host species.

1.2 Evidence for density-dependent transmission of rabies

It is difficult to determine the direct relationship between disease incidence, host density and transmission under field conditions, particularly for wildlife given their inaccessibility (Wandeler *et al.* 1974b; Macdonald & Voigt 1985; Beyer *et al.* 2010). Consequently, we are left with interpreting indirect and somewhat conflicting evidence regarding the role of density

in rabies transmission in wildlife and dogs. In this section we examine four key lines of evidence about the functional forms of rabies transmission.

1.2.1 Cycles in disease incidence

Cycles in disease incidence have motivated some of the most effective applications of population modelling in ecology (Anderson & May 1991; Begon, Harper & Townsend 1996). Mathematical models can explore how different biological hypotheses relate to the expected amplitude and period of cycles, providing insights into the drivers of transmission. Perhaps, the most successful examples of this have been in the study of childhood infectious diseases (Earn *et al.* 2000; Altizer *et al.* 2006) where detailed historical records have allowed the application of sophisticated methods of statistical inference (Bjornstad, Finkenstadt & Grenfell 2002; Grenfell, Bjornstad & Finkenstadt 2002). However, even in the absence of detailed data, models can provide useful insights simply through the ability of a given mechanism to generate periodic dynamics.

Cycles have been observed for wildlife (Friend 1968; Bogel *et al.* 1974; Childs *et al.* 2000; Courtin *et al.* 2000; MacInnes *et al.* 2001) and canine rabies (Ernst & Fabrega 1989; Bingham *et al.* 1999a; Widdowson *et al.* 2002; Hampson *et al.* 2007), although periodicity in incidence is not a consistent finding (Macdonald & Voigt 1985; Zinsstag *et al.* 2009). The mechanistic driver of these cycles is widely assumed to be the interaction of density-dependent transmission, rabies-induced mortality and other demographic processes (Bogel *et al.* 1974; Steck & Wandeler 1980; Anderson *et al.* 1981; Childs *et al.* 2000; Hampson *et al.* 2007). However, it is important to determine if this assumption is correct given its implications for culling.

Deterministic compartmental models have been used to describe rabies dynamics in wildlife (Anderson *et al.* 1981; Coyne, Smith & McAllister 1989) and domestic dogs (Cleaveland & Dye 1995; Coleman & Dye 1996; Kitala *et al.* 2002; Hampson *et al.* 2007; Carroll *et al.* 2010). These models assume random mixing, neglecting the spatial and social heterogeneity that exists in real populations. Within such "well-mixed" models, frequency-dependent transmission of fatal diseases inevitably leads to rapid die-out of the host population (Keeling & Rohani 2008). Under frequency dependence, the average reproductive potential of the pathogen is unchanged during the spread of an epidemic. With no mechanism to arrest the spread of disease, transmission continues and the host and parasite populations go extinct. In contrast, under the assumption of density-dependent transmission, epidemics will subside

when the host density falls below the invasion threshold (where $R_0 = 1$). The time delay between epidemic peak and replenishment of the host population generates damped epidemic cycles through delayed density dependence. The assumption of density-dependent transmission is therefore the most parsimonious mechanism by which stable epidemic cycles for rabies can be supported within deterministic random mixing models. However, in structured populations, epidemic cycles may be generated by alternative mechanisms even when the transmission rate is frequency-dependent.

Age structure is one such potential mechanism. Attack rates for rabies appear to vary considerably with age, with reported incidence in foxes in Europe (Wandeler *et al.* 1974b) and raccoons in Ontario (Rosatte *et al.* 2006) concentrated within adult age classes. Within an age-structured model, the net reproductive ratio of rabies will not only depend on the rates of transmission, but also on the age-distribution in the population (Anderson & May 1991). If the basic reproductive ratio is only above unity for a core-group of high-risk individuals, the epidemic can recede when this core-group is exhausted. The delay between depletion of the core group and replenishment through births can generate cycles in incidence that may be sustained by seasonal birth pulses (Davis & Wood 1959; Lloyd *et al.* 1976).

Deterministic thresholds are not the only possible mechanism by which endemic co-existence of rabies could be maintained within frequency-dependent transmission models. An important limitation of deterministic models is that they do not account for the probability of local extinction of disease following an epidemic. In areas where rabies in foxes is not actively controlled, 3-4 yearly cycles in incidence are observed at regional levels [around 1000 km^2 in Europe and at the county level in Canada] (Johnston & Beauregard 1969; Bogel *et al.* 1974), and are out of phase between regions (Johnston & Beauregard 1969; Bogel *et al.* 1974; Macdonald & Voigt 1985). Epidemics have been associated with considerable reductions in host populations by up to 50% (Bogel *et al.* 1974). This reduction in the density of the host species within a region and the corresponding reduction in the instantaneous numbers of infective individuals will increase the chances of rabies becoming locally extinct before the host population is exhausted. Stochastic population thresholds for persistence of rabies can exist irrespective of the mode of transmission (Lloyd-Smith *et al.* 2005). Stochastic extinction and re-introduction of rabies following the local re-structuring of host populations (Steck & Wandeler 1980; Anderson *et al.* 1981; Macdonald & Voigt 1985), consistent with metapopulation dynamics, are also viable alternative mechanisms to generate these dynamics.

In conclusion, cycles in rabies incidence observed in wildlife could be supported by density- or frequency-dependent transmission when stochasticity and the heterogeneous structure of real populations are accounted for.

Although deterministic density-dependent models have been used to describe rabies dynamics in domestic dogs, reactive vaccination can also drive cycles in incidence (Hampson *et al.* 2007). For example, in Zimbabwe between 1950 and 1995, the amplitude and interval of peaks in rabies varied (from 75 to 350 cases per year and inter-epidemic periods from 4 to 20 years) with the level of vaccination delivered during national vaccination campaigns (Bingham *et al.* 1999a). These observations provide little insight into the processes driving local disease dynamics for dogs. Rather, other evidence for the functional forms of transmission of canine rabies will be considered in the next sections.

1.2.2 The relationship between R_0 and host density

As discussed above, R_0 is expected to increase with density for density-dependent transmission and remain constant irrespective of density for frequency-dependent transmission. R_0 may be estimated from the (exponential) rate of growth early in an epidemic prior to significant susceptible depletion or implementation of control measures (Heffernan, Smith & Wahl 2005; Wallinga & Lipsitch 2007). Using this method, Hampson *et al* (2009) obtained estimates of R_0 for canine rabies, across a wide geographical range, of between 1.05 and 1.72. The range of these estimates is similar to the statistical uncertainty in simulated epidemics when the biting behaviour of rabid dogs is accounted for. Dog population densities were reported for only four of these locations, ranging from 1.36 dogs km^{-2} in rural Tanzania to 110 unrestricted dogs per km^2 in urban Mexico. However, other locations cited in the study are likely to represent even higher densities, with the highest reported density in the general literature being 2388 dogs km^{-2} in Guayaquil, Ecuador (Beran & Frith 1988). The absence of any correlation between R_0 and host density across such a large range of densities is consistent with earlier studies (Coleman & Dye 1996; Kitala *et al.* 2002) and suggests that if a relationship between transmission and dog density does exist, it must be quite weak.

Equivalent data are not available for wildlife. Compared to canine rabies, incidence records generally have a lower temporal resolution (typically quarterly or annually) (Macdonald & Voigt 1985; Rhodes *et al.* 1998; Bingham *et al.* 1999b; Rosatte *et al.* 2006), and the ranges of host densities are narrower: 0.8-1.2 jackals km^{-2} during the breeding season on commercial farmland in Zimbabwe (Rhodes *et al.* 1998), 5.4-9.1 racoons km^{-2} (averaged over a four year

period) for rural Ontario (Rosatte *et al.* 2007) and 0.5-1.8 adult foxes km^{-2} in central Europe (Lloyd *et al.* 1976).

This apparent lack of relationship between R_0 and host density is most consistent with frequency-dependent transmission. However, as previously discussed, random mixing models with frequency-dependent transmission of rabies predict host extinction as soon as R_0 exceeds unity. This prediction is inconsistent with the very low attack rates reported for canine rabies compared to wildlife rabies and with the absence of large declines in population densities from rabies-induced mortality (Hampson *et al.* 2007). Estimates of the incidence, or average monthly attack rates, are typically below 0.5% and rarely exceed 2% (Waltner-Toews *et al.* 1990; Windiyaningsih *et al.* 2004; Zinsstag *et al.* 2009; Tenzin *et al.* 2010; Putra *et al.* 2011; Tenzin *et al.* 2011).

This incongruity between attack rates and the apparent scaling of R_0 may be resolved by considering a more complex relationship between rabies dynamics in dogs and anthropogenic factors than has previously been assumed. Suspect rabid and in-contact dogs are often identified and killed swiftly by the community (Hampson *et al.* 2007; Hampson *et al.* 2009), a practice hereafter referred to as 'selective removal'. This reduces the effective infectious period in dogs (Hampson *et al.* 2009) and could contribute to the relatively lower incidence as compared to wildlife. The selective removal of infectious and in-contact dogs was thought to have contributed to the control of rabies in eastern Bhutan (Tenzin *et al.* 2011) and the United Kingdom (Pastoret & Brochier 1998). Indeed, euthanasia (WSPA 2012) of infected dogs is advocated to control rabies (WHO 2004). Such behavioural responses to the spread of epidemics are rarely considered in epidemiological models (Ferguson 2007; Funk *et al.* 2009) but are likely to play a particularly important role in disease transmission within owned, and managed, populations. Selective removal may conceal the existence of density-dependent transmission processes if the rate of intervention also scales with density.

We thus hypothesize that selective removal itself might be density-dependent for several reasons. First, rabid dogs may be more quickly spotted and selectively removed from areas with more people present. Second, given that most dogs are owned (WHO & WSPA 1990; Cleaveland & Dye 1995; Butler & Bingham 2000; Windiyaningsih *et al.* 2004), dog and human population densities are expected to correlate (Oboegbulem & Nwakonobi 1989; Matter *et al.* 1998; Butler & Bingham 2000). Finally, other anthropogenic factors that may interfere with contact processes, such as traffic or urban infrastructure, are also likely to scale with human and dog density. Therefore, the effective infectious period, as reduced by

selective removal, could scale inversely with human, and thus dog, population density. The estimates of R_0 discussed above are conditional on the assumption of a fixed infectious period. Any systematic variation in the infectious period with population density could counteract the impact of density-dependent contact rates and result in R_0 appearing density-independent. Under this hypothesis, density-dependent transmission could not be ruled out unequivocally for canine rabies.

As a final consideration, stochastic fade-out is expected with low attack rates. However, rabies often appears to persist in dog populations. This may be because selective removal and stochastic processes are off-set by the continual translocation of dogs (some of them infected) by people (Beran & Frith 1988; Denduangboripant *et al.* 2005; Coetzee & Nel 2007; Kasempimolporn, Jitapunkul & Sitprija 2008; Zinsstag *et al.* 2009) consistent with metapopulation dynamics (Hanski & Gaggiotti 2004; Beyer *et al.* 2010). In conclusion, more intensive study of the mechanisms underlying rabies transmission and persistence in domestic dog populations is warranted to understand these empirical patterns.

1.2.3 Thresholds for invasion and increasing incidence with population density

The existence of a threshold in host population density below which infection cannot spread (i.e. where $R_0 < 1$) would be direct evidence in support of density-dependent transmission. Such invasion thresholds in wildlife and domestic dog populations have been proposed based on a limited number of studies that compared disease incidence between different geographic locations with different host densities (Steck & Wandeler 1980; Beran & Frith 1988; Cleaveland & Dye 1995). However, as discussed below, it is not possible to establish the relationship between host density and disease incidence based on these data.

Threshold densities for invasion have been suggested to occur where canine rabies is observed to change from sporadic disease at lower densities to persistence at higher densities (Beran & Frith 1988; Cleaveland & Dye 1995). However, these observations could also be explained by increased stochastic fade-out of disease at lower densities where there are lower numbers of infected dogs. In general, the probability of stochastic fade-out will decrease with an increase in R_0 or in the number of infected individuals (Lloyd-Smith *et al.* 2005). This effect may be particularly relevant to dogs where more infected individuals may be introduced into larger or more dense populations by people (Denduangboripant *et al.* 2005; Kasempimolporn, Jitapunkul & Sitprija 2008; Zinsstag *et al.* 2009). Consequently, the probability of stochastic fade-out is predicted to decrease with an increase in population size

or density. Even when R_0 is invariant between populations of different sizes or densities, stochastic effects may give the impression of a deterministic threshold for invasion where one does not exist. This is particularly likely when R_0 is low. Should a deterministic threshold for invasion exist, it may be obscured by these processes and be lower than estimated empirically.

The key data used to support the existence of a threshold density in foxes are expressed in terms of the hunting indicator of population density (HIPD) (Steck & Wandeler 1980). HIPD is an indirect estimate of density, with well-known biases (Wandeler 1980; Macdonald & Voigt 1985). However, there are two specific issues with the use of these data to support a threshold density for fox rabies. First, HIPD estimates below the purported threshold density for invasion were not recorded, thus precluding any conclusion of an invasion threshold. Second, the observed positive correlation between the annual number of animal rabies cases per km^2 per year and the HIPD has been wrongly interpreted as evidence for density-dependent transmission. Assuming the HIPD correlates with host density, such a relationship would be expected whether transmission depends on fox density or not. Determining the mode of transmission would require an evaluation of disease incidence as a proportion of the total population size or density (Rothman, Greenland & Lash 2008), which cannot be inferred from HIPD.

1.2.4 Impacts of density reduction

Density reduction, particularly culling (i.e. the widespread killing of hosts regardless of infection status), has been undertaken to reduce the incidence of rabies and therefore eliminate the disease on the basis that transmission is density-dependent. As previously discussed, the assumption of density dependence originates from the interpretation of cycles in wildlife rabies and thresholds for invasion for foxes and dogs. However, the fact that culling has failed to achieve sustained control of rabies in wildlife and dogs (Kaplan, Goor & Tierkel 1954; Anderson *et al.* 1981; Macdonald & Voigt 1985; Anderson 1986; Beran & Frith 1988; WHO 2004; Windiyaningsih *et al.* 2004; Cleaveland *et al.* 2006) may be the best evidence that a simple relationship between disease incidence and host population density does not exist for rabies. We now discuss evidence from culling programmes (dogs and wildlife) followed by more limited evidence on sterilization campaigns.

1.2.4.1 Culling

Culling has been shown to be ineffective in controlling rabies in all host species. Rabies persisted in foxes in New York State despite 'concentrated reduction campaigns' following an outbreak in 1945, while simultaneous vaccination of dogs in the State eliminated rabies from this species (Friend 1968). Similarly, in Denmark in 1964, culling did not prevent rabies outbreaks in foxes; however, rabies did not occur where dogs in the same region had been vaccinated (Muller 1966; Muller 1971). In response to a rabies outbreak in 1997 nearly 300,000 dogs, approximately half of the population estimated at the start of the outbreak, were culled in Flores, Indonesia over a period of 4 years. However, in 2004 rabies was still endemic although the total dog population was still considerably reduced (Windiyaningsih *et al.* 2004). Culling failed to control canine rabies in Korea (Lee *et al.* 2001) and Israel (Kaplan, Goor & Tierkel 1954), whereas subsequent vaccination in both countries controlled the disease.

Culling has been used to control ongoing outbreaks and to prevent the invasion of rabies in foxes. Declines in rabies cases have followed outbreaks irrespective of active culling (Bogel *et al.* 1974), with stochastic extinction expected (Anderson *et al.* 1981) particularly where disease-induced mortality is substantial (Bogel *et al.* 1974). Within a given area, culling might be expected to amplify these processes, increasing the probability of stochastic extinction regardless of density dependence. Indeed, rabies appeared to die-out in some areas where fox dens were gassed (Wandeler *et al.* 1974b). However, the limited data available are unclear regarding how culling interacts with disease-induced mortality during an epidemic and how it may change disease dynamics (Wandeler *et al.* 1974b). Other processes may also counter the effect of density reduction on disease incidence. Examples include social perturbations, as demonstrated in badger populations (Woodroofe *et al.* 2006a; Woodroofe *et al.* 2006b), and interactions between the level of culling, age structure (Bolzoni, Real & De Leo 2007) and demographic processes (Choisy & Rohani 2006).

Culling has also failed to prevent outbreaks of rabies in foxes in previously unaffected areas or the recurrence of the disease in areas where it had died-out, as observed in southern Denmark (Muller 1971). Where density-dependent transmission has been assumed, invasion thresholds are reported to vary and to be low (i.e. <1 fox km^{-2} in Europe and <0.4 foxes km^{-2} in Ontario). Thus, even if transmission were density-dependent, reductions in density to below an invasion threshold may not be achievable practically or be sustainable (Wandeler *et al.* 1974a; Anderson *et al.* 1981).

Culling has generally failed to eliminate outbreaks of rabies in dogs. In our review of the scaling of rabies transmission rates with density (in the previous sections) we have found no conclusive evidence to support either the frequency-dependent or density-dependent assumption for canine rabies. We are therefore unable to unequivocally conclude that the ineffectiveness of culling is because transmission is frequency-dependent. An alternative explanation is that reductions in densities to below invasion thresholds are not achievable practically. Canine rabies can circulate where densities are as low as 1.36 dogs km^{-2} (Hampson 2009), which is substantially lower than the densities reported for most free-roaming dog populations. Under the assumption of density-dependent contact rates, culling and vaccination should have similar impacts on disease incidence. Thus, given estimated values of $R_0 < 2$, control should be achieved by culling at most half the population. Yet, in Flores, Indonesia, rabies persisted after this level of culling was achieved (Windiyaningsih *et al.* 2004). More generally, the stochastic persistence of canine rabies despite low attack rates and considerable density reduction is interesting irrespective of the mode of transmission.

The fact that rabies often persists despite culling may be a function of human factors. The continual translocation of dogs (some infected) with people (Beran & Frith 1988; Denduangboripant *et al.* 2005; Coetzee & Nel 2007; Kasempimolporn, Jitapunkul & Sitprija 2008; Zinsstag *et al.* 2009) may off-set the selective removal of infectious and in-contact dogs and stochastic extinctions. Where culling occurs simultaneously, translocation may also off-set any reductions in the incidence of rabies. In addition, translocation may be exacerbated in response to culling campaigns. For example, within a few days of a village-wide cull in Kelusa, Bali, where rabies had not occurred previously, two residents brought in unvaccinated, potentially infected puppies from outside the village to replace their culled, vaccinated adult dogs. As attack rates are typically very low, culling predominately removes healthy dogs, and some of these may be vaccinated and hence unlikely to become infected. Other compensatory mechanisms may also off-set reductions in host density. These include concomitant reductions in mortality from reduced competition for food (although the actual intensity of competition in free-roaming dogs is unknown), reductions in the dumping of surplus puppies/unwanted dogs and improved care of dogs. To address these issues, we are currently investigating the effects of human behaviour in response to culling on dog population dynamics and disease transmission in Kelusa.

The ethics of culling healthy, free-roaming animals in conjunction with vaccination programmes are also debatable. Raccoons have been culled on Wolfe Island, Ontario, as a means to reduce the number of animals that needed to be trapped and vaccinated (Rosatte *et*

al. 2007). The same justification may be extended to dogs, and a variable degree of culling of free-roaming dogs, historically regarded as 'strays', has often been undertaken alongside mass vaccination programmes (Wells 1954; Cheuk 1969; Larghi *et al.* 1988; Ernst & Fabrega 1989). However, despite appearances, the vast majority of free-roaming dogs in most societies globally are owned (WHO & WSPA 1990; Cleaveland & Dye 1995; Butler & Bingham 2000; Windiyaningsih *et al.* 2004) and in reasonable health. Not only are these dogs more accessible to vaccination than commonly recognised, but culling healthy animals can result in unintended negative consequences on both animal welfare and disease control.

1.2.4.2 Sterilization

The use of immunological and chemical sterilization has been modelled for the control of rabies in wildlife and in dogs (Suppo *et al.* 2000; Smith & Cheeseman 2002; Carroll *et al.* 2010). However, only surgical sterilization has been used in dogs under field conditions. Sterilizations are usually carried out by nongovernmental organizations and local authorities, which aim to vaccinate and simultaneously sterilize at least 70% of the dog population (Totton 2009). Limited data suggest that these programmes reduce the incidence of rabies and may stabilize or gradually reduce population density over time-scales of several years (Reece & Chawla 2006; Totton 2009; Totton *et al.* 2010). However, the respective impacts of vaccination and sterilizations have not been assessed. Reductions in population density may plausibly reduce the number of dogs that require vaccination, although timely reductions in density may be constrained by resources and population dynamics (Hemachudha 2005). As with culling, the demand for dogs by communities may result in an increase in dog importation where local supply has been reduced by sterilization. Thus, we are studying the effect of human behaviour in response to sterilization on dog population dynamics and disease transmission in Antiga, Bali.

1.3 Conclusion

There is still considerable uncertainty surrounding the role of density in the transmission of rabies in animal host species. Density has been assumed to be the key factor that drives transmission, with important implications for the use of population reduction as a means to control rabies. However, it is evident that the relationship between host density, disease incidence and other factors is complex and varies between species. Further research to determine the factors that drive rabies transmission would not only enhance development of

epidemiological models but also inform the development of effective, sustainable disease control measures.

Determining the effect of density in the transmission of rabies in wildlife hosts is constrained by the lack of high-resolution data exhibiting sufficient variability in both disease incidence and host densities. We have discussed how cycles in the incidence of rabies in foxes and raccoons can occur under either frequency- or density-dependent transmission, and how both model structures could account for the failure of culling to control rabies.

Although still limited, better quality data for dogs suggests a more complicated relationship between contact rates and host density. The evidence indicates that not only is reducing dog density ineffective at controlling rabies, but culling in particular often has unintended negative consequences. We advocate more systematic investigation of the human factors that could affect the dynamics of rabies in dogs, to understand possible contrasts with the situation in wildlife.

In contrast to culling, vaccination programmes against rabies in dogs (Cleaveland *et al.* 2003; WHO 2004; Schneider *et al.* 2005; Cleaveland *et al.* 2006; Davlin & VonVille 2012) and wildlife (Wandeler *et al.* 1988; Brochier *et al.* 1991; MacInnes *et al.* 2001; Rosatte *et al.* 2007) have proven efficacy and feasibility across a wide range of settings and raise far fewer ethical or welfare issues.

Chapter 2

The demography of free-roaming dog populations and applications to disease and population control

Summary

Understanding the demography of domestic dog populations is essential for effective disease control, particularly of canine-mediated rabies. Demographic data are also needed to plan effective population management. However, no study has comprehensively evaluated the contribution of demographic processes (i.e. births, deaths and movement) to variations in dog population size or density, or determined the factors that regulate these processes, including human factors. We report the results of a three-year cohort study of domestic dogs, which is the first to generate detailed data on the temporal variation of these demographic characteristics. The study was undertaken in two communities in each of Bali, Indonesia and Johannesburg, South Africa, in rabies endemic areas and where the majority of dogs were free-roaming. None of the four communities had been engaged in any dog population management interventions by local authorities or animal welfare organizations. All identified dogs in the four communities were monitored individually throughout the study.

We observed either no population growth or a progressive decline in population size during the study period. There was no clear evidence that population size was regulated through environmental resource constraints. Rather, almost all of the identified dogs were owned and fed regularly by their owners, consistent with population size regulated by human demand. Finally, a substantial fraction of the dogs originated from outside the population, entirely through the translocation of dogs by people, rather than from local births. These findings demonstrate that previously reported growth of dog populations is not a general phenomenon and challenge the widely held view that free-roaming dogs are unowned and form closed populations. These observations have broad implications for disease and population control. The accessibility of dogs for vaccination and evaluation through owners and the movement of dogs (some of them infected) by people will determine the viable options for disease control strategies. The impact of human factors on population dynamics will also influence the feasibility of annual vaccination campaigns to control rabies and population control through culling or sterilization. The complex relationship between dogs and people is critically important in the transmission and control of canine-mediated rabies. For effective

management, human factors must be considered in the development of disease and population control programmes.

2.1 Introduction

Understanding the demography of domestic dog populations in the developing world is critical for planning effective population management and disease control, particularly for rabies which causes around 55,000 human deaths per year (Knobel *et al.* 2005), as well as for other dog-mediated zoonoses prevalent in developing countries (Macpherson, Meslin & Wandeler 2012). Most human rabies cases are caused by bites from dogs infected with this fatal encephalitic disease. Rabies is directly transmitted by bites between dogs and can be readily controlled by canine vaccination (Jackson 2013). Demographic processes (i.e. births, deaths and the movement of dogs into and out of populations) contribute to variations in population size, density, vaccination coverage and disease transmission. A number of studies, encompassing a range of geographic locations, have assessed aspects of canine demography, including longitudinal studies where population management was not systematic (Chomel *et al.* 1987; Kitala *et al.* 2001; Pal 2001; Hampson *et al.* 2009). However, the contributions of demographic processes to population and disease dynamics, and the factors that regulate these processes, have not been comprehensively investigated.

Several studies have estimated variations in the size of dog populations, where most dogs are free-roaming and where there has been no population control (Brooks 1990; Butler & Bingham 2000; Kitala *et al.* 2001; Pal 2001; Hampson *et al.* 2009; Acosta-Jamett *et al.* 2010; Gsell *et al.* 2012). These all report population growth, but with the exception of Pal (2001), growth was determined indirectly from estimates of births and deaths and age structure in a sub-set of dogs, or extrapolated from human census data and dog to human ratios. Rapid declines in vaccination coverage, necessitating at least annual vaccination campaigns, were also determined from similar data (Brooks 1990; Kitala *et al.* 2001; Hampson *et al.* 2009; Acosta-Jamett *et al.* 2010; Gsell *et al.* 2012). The effect of movement on these variations was not considered.

There is increasing evidence that most free-roaming dogs are owned (Cleaveland & Dye 1995; Butler & Bingham 2000; Windiyaningsih *et al.* 2004; Gsell *et al.* 2012). Movement of dogs by people may therefore contribute to population dynamics (Chomel *et al.* 1987; Beran & Frith 1988), the spread of rabies (Denduangboripant *et al.* 2005; Zinsstag *et al.* 2009; Talbi *et al.* 2010; Townsend *et al.* 2013b), and off-set the impact of population control programmes

that aim to reduce population density through culling (Beran & Frith 1988; Windiyaningsih *et al.* 2004) or sterilization (Totton *et al.* 2010).

In canine epidemiological and ecological models, it is often implicitly assumed that free-roaming dog populations are regulated through environmental resource constraints on births and deaths (Kitala *et al.* 2002; Hampson *et al.* 2007; Zinsstag *et al.* 2009; Totton *et al.* 2010). This has been described in rabies control policy by the logistic growth model (Wandeler 1985; WHO & WSPA 1990); a simple model that assumes all individuals have equal access to resources at the population level and births and deaths vary linearly and uniformly with population density, a surrogate for environmental resource availability (Sibly & Hone 2002; Vandermeer & Goldberg 2003). While the overall contribution of resource availability to variations in vital rates is controversial, empirical evidence suggests that it is important in a range of feral ungulate and wildlife populations (Choquenot 1991; Albon *et al.* 2000; Coulson, Milner-Gulland & Clutton-Brock 2000; Coulson *et al.* 2001; Bonenfant *et al.* 2002; Coulson *et al.* 2004). The relationship between density and births and deaths is demonstrable in these populations because of large fluctuations in population density about a carrying capacity, K. These fluctuations are either intrinsic or the result of deliberate perturbations, i.e. harvesting. Simple models assume that as populations approach or exceed K, all individuals in the population uniformly experience the effects of resource depletion. In reality, the relationship between density and births and deaths is complex and may be non-linear with greatest change in births and deaths at or near K (Fowler 1981), and resource depletion primarily affecting those individuals with the highest nutritional requirements, e.g. the survival of rapidly growing juveniles (Clutton-Brock, Major & Guinness 1985; Coulson *et al.* 2001; Bonenfant *et al.* 2002).

The assumption that environmental resource constraints regulate dog populations has never been properly investigated. In accordance with the logistic growth model, it implies the primary food source of free-roaming dog populations is available to all dogs and most likely to be environmental refuse, and that dog populations are self-sustaining and population size and vital rates are self-regulating; effectively the dogs are unowned. This contradicts increasing evidence that most free-roaming dogs are owned (Cleaveland & Dye 1995; Butler & Bingham 2000; Windiyaningsih *et al.* 2004; Gsell *et al.* 2012) with serious implications for disease and population control measures and for animal welfare. However, determining the relationship between dog population density and births and deaths is hampered by the lack of longitudinal data from dog populations with substantial variations in density. Although dog populations are frequently culled, culls are often non-systematic and difficult to monitor,

which complicates assessment of the role of environmental resource constraints in regulating free-roaming dog populations. Furthermore, similar to ecological studies of feral ungulates and wildlife, quantifying variations in the distribution, volume, nutritional content and, more critically, uptake of environmental resources is generally not practicable. However, the unique relationship between dogs and humans affords a multifaceted approach, combining community-based methods with direct observations to infer the mode of ownership and food sources for individuals.

Using this multifaceted approach, we directly measured temporal variations in population size and the contributions of births, deaths and human-mediated movement on these variations. We also investigated the effect of human and other factors, including environmental resource constraints, associated with these demographic processes. To facilitate comparisons between different environments and cultures, two populations of free-roaming dogs were selected in Bali, Indonesia, and two in Gauteng Province, South Africa, where rabies is endemic and where free-roaming dogs have been assumed to be unowned. We found constant or declining population sizes with no clear evidence of population regulation by environmental resource constraints; that almost all identified dogs in the communities were owned and fed regularly by their owners, consistent with population size regulated by human demand; that a substantial fraction of dogs originated from outside the population; and, that high levels of vaccination coverage may afford protection from rabies for up to two years.

2.2 Methods and materials

2.2.1 Research sites

Data were collected from four communities, two in South Africa and two in Indonesia. The sites were selected during preliminary visits to Johannesburg and Bali in 2007 based on criteria including community support for the study, the absence of previous dog population management interventions by local animal welfare non-governmental organizations (NGOs) or authorities, geographic accessibility, operator safety and the availability of NGO support for data collection and translation. Rabies outbreaks occurred in Bali in 2008 and Gauteng Province in 2010.

The two sites in Gauteng Province, South Africa comprised the informal settlement Zenzele west of Johannesburg (26.15^{o}S and 27.41^{o}E) and Braamfischerville in Soweto (26.12^{o}S and 27.52^{o}E). The study area encompassed the entire Zenzele township, whereas approximately

one third of Braamfischerville was included in the study area to include a comparable number of dogs to Zenzele. In Indonesia, the two sites included the villages of Kelusa (8.26^{o}S and 115.15^{o}E) and Antiga (8.30^{o}S and 115.29^{o}E) on the island of Bali. In Kelusa, the study area encompassed the entire village with the exception of Banjar Yehtengeh, which is separated from the rest of the village by rice fields and jungle, the southern half of Banjar Kelikikawan and the entrances (i.e. a typical compound housing extended families) scattered along the main road leading into the village. In Antiga, the study area encompassed all of the main residential area (Banjars Kaler and Kelod). An additional area within Banjar Ketug included entrances scattered along a 2.7 km stretch of road winding through the jungle north of Kaler and Kelod.

All households in the study areas were included in the sampling frame. In Zenzele the sample unit, or household, is a systematically numbered yard with usually one or two shacks and poor fencing. In Braamfischerville, a household is a systematically numbered yard with a small, fixed structure, and a variable number of shacks and variable quality fencing. In Bali, a household is equivalent to an "entrance" associated with variable quality fencing. Given the lack of street names and house numbers, entrances were identified by photograph. With the exception of Zenzele, the study areas were established with no new households built during the study period. In May 2009 one new street of shacks was erected at the north end of Zenzele. Antiga and Zenzele are of comparatively lower socioeconomic status, and Banjar Ketug and Zenzele are without household sewage systems or water supply.

2.2.2 Method and type of data collected

Individual-level data for every identified dog in the study area were collected longitudinally by direct observation and questionnaire from March 2008 until April 2011. The study population comprised of every owned dog (i.e. dog belonging to a household in the study area). Each owned dog was included in the study population immediately upon identification at its household, and recorded by photograph (standardised dorsal and lateral views) and owner questionnaire and visually assessed (see below for more details). Pups were recorded but not photographed until their third month of life. Dogs were not photographed consistently during 2010 because handling that occurred due to rabies vaccination campaigns in that year (as part of the same research project) caused them to become flighty and difficult to photograph and because by that time the primary researcher and enumerators were familiar with the majority of dogs. Each dog in the study population was individually recognisable and monitored at its household through direct observation, by the primary researcher and

enumerators, and questionnaire for the remainder of the study period or until it was lost from the study area.

Households were visited during door-to-door censuses undertaken every 3-4 months (3-5 months in Braamfischerville) during the study period. The first inter-census period was longer (Zenzele: ~5 months, Braamfischerville: ~8 months and Kelusa: ~ 4.5 months). Eleven censuses were undertaken in Zenzele and ten in Braamfischerville, ranging from 12-23 days and 16-31 days to complete respectively. Nine censuses were undertaken in each of Kelusa and Antiga, ranging from 8-16 days and 11-19 days to complete respectively. All households with female dogs were revisited by the enumerators between all the censuses. Therefore, every household in the study area was visited frequently during the study period and most owned dogs were observed directly by the primary researcher and enumerators. However, a proportion of dogs were owned transiently between household visits and were not observed directly by the research team; these dogs were also recorded by owner questionnaire. These were generally young dogs that were acquired and then died between successive visits. Households were visited on foot during daylight hours and in approximately the same order.

In addition to owned dogs being monitored at their household, every dog encountered in a yard not their own or on the street during each census was identified by the primary researcher and enumerators as either belonging to a household in the study area or not. Each dog identified as not belonging to a household in the study area was classified as unowned. A description of the unowned dog was recorded and, whenever possible, the dog was photographed. Only two dogs in Johannesburg and eighteen dogs in Bali were identified as unowned; these dogs were not included in the study population but are reported separately (see Results section 2.3.5).

Focus groups with community leaders, members and enumerators, including participatory rural appraisal (PRA) techniques (Kumar 2007), were undertaken in Zenzele during February 2008 to a. investigate the ecology of the dog population in the study area, and b. develop the questionnaires. See Appendix 2.1 for a description of questionnaire development and implementation. Information collected, by direct observation and questionnaire, for every identified owned dog during each census and revisit included: house number, dog's name, age, gender, source, outcome (e.g. died, relocated), reason for ownership, physiological, clinical and (for females) reproductive status, nutrition (type and source), body condition score (BCS) and level of confinement. Body condition score was a surrogate for food volume given the practical limitations of quantifying food uptake from all possible sources, including

owners. The dogs in Bali could not be readily handled. Therefore, a standard 9-point scoring system (emaciated score 1 to obese score 9) validated in adults (Laflamme 1997) and modified to assess body condition score without palpation was used. The modified system had been validated using dual energy x-ray absorptiometry in 71 dogs, including a small number of growing dogs (German & Holden 2006; German *et al.* 2006). Each dog, generally in its third month of life or older, received two independent body condition scores from the primary researcher and enumerators during each census or revisit. Clinical examinations were undertaken during each census by the primary researcher, a qualified veterinarian. The date and age of dogs at acquisition was reported by owners and/or visually assessed, including from the dentition of pups and juveniles (Dyce, Sack & Wensing 1987) in Johannesburg. For most dogs, these data were re-recorded at least once during the study period. The date a dog was lost from the study area was generally only recorded once. The month of loss was reported by the owner for 68% of the lost dogs in Zenzele, 78% in Braamfischerville, 51% in Kelusa and 55% in Antiga. Except where stated (see Table 2.4), the remainder were assumed to be lost uniformly between the census or revisit in which they were last recorded and the subsequent one. Apart from the primary researcher, all enumerators were local residents employed by NGOs. Data collection was standardised through detailed enumerator training at the start of the study and repeated on the first day of each census.

All known refuse in the study areas was evaluated non-systematically by the primary researcher. Refuse was photographed periodically, and its distribution and the presence of edible organic matter assessed subjectively at each visit.

Participatory approaches were preferred to mark-recapture or more technically demanding surveillance techniques, such as monitoring the movement of dogs with GPS collars, to further investigate the presence of a resident population of healthy, unowned dogs (i.e. dogs not belonging to households in the study area or outside the study area) in the Bali villages. Measurement error and statistical variation, violations of mark-recapture model assumptions and the need for repeat photographic mark-recapture preclude the use of these techniques to identify a real number of unowned, healthy dogs resident in the population, particularly where this sub-group is likely to be small. On this basis, participatory exercises were undertaken from April 2011 – April 2012 and are reported separately (Chapter 3) .

2.2.3 Analytical methods

For the analysis pups are defined being in their 1st – 3rd month of life (i.e. 0 – ~13 weeks of age), juveniles 4th – 12th month of life (i.e. ~14 – 52 weeks of age), and adults older than their 12th month of life. Owned dogs were included in the analysis once they reached their third month of life (i.e. approximately 8 weeks of age) (here after referred to as "registered" dogs). Pups born in households in the study area but lost before their third month of life and unowned dogs are reported separately.

Non-parametric regression was used to explore trends in population size, mortality and pregnancy. Visual inspection of plots and autocorrelation and partial-autocorrelation were used to assess periodicity, particularly seasonality, for these variables. An extended data set (i.e. by an additional 5 months) for Antiga was available.

The proportion of reproductively mature females pregnant per month was estimated to avoid variation in pregnancy confounded by any seasonal variation in population size, most likely from disease-induced mortality such as babesiosis which has a reported seasonal distribution (Collett 2000). Bitches continually confined to a dog proof yard were excluded from these analyses. Monte Carlo estimates of the proportion of females in early (i.e. not visible) pregnancy when lost from the study populations were obtained by sampling from the observed distributions of age at first pregnancy and interval between the first and subsequent litters. Mortality was also estimated as the proportion of dogs dying per month and in terms of total mortality, specific disease-induced mortality and "other" (i.e. disease-induced mortality and dogs found dead, missing entries and unknown causes).

We used Cox proportional hazard models to evaluate the risk of loss from the starting cohorts by age class at the start of the study and by gender. To model declines in vaccination coverage, estimates of vaccination coverage were obtained by assigning a random sample of dogs from the starting cohort equal in size to the proportion assumed to be vaccinated, and determining those still present at 12 and 24 months. This process was repeated 1000 times to produce Monte Carlo estimates of vaccination drop-off. A Bayesian ordinal regression framework (McKinley, Morters & Wood 2014) was used to investigate whether there were clear trends between body condition and increased caloric requirements from growth and lactation (National Research Council 2006). All observations with complete information for the variables of interest (Appendix 2.26) were included in the analyses. To account for observer variability, we fitted four versions of the model using minimum or maximum BCS (between observers) as response variables, and two definitions of gestation and lactation

(estimated [63 days gestation and 12 weeks lactation] and observed). Analysis was repeated without the first time point (i.e. censuses March-May 2008) to allow for owner reported clinical signs for the previous three months (see Appendix 2.1). We tested for an association between population size and births and deaths.

Data analyses were conducted using R (R Core Team 2014) and C.

2.3 Results

2.3.1 Study population

Throughout the study there was a very high level of compliance, with a low rate of partial- and non-respondents (Zenzele 1.5%, Braamfischerville 1.2%, Kelusa 2.5% and Antiga 2.2%). While some respondents declined to complete the full questionnaire, all provided partial information, such as the number, source and outcome, and permitted visual assessment of their dogs periodically throughout the study period. A total of 3240 owned dogs were registered during the study period: 1022 in Zenzele, 882 in Braamfischerville, 707 in Kelusa and 629 in Antiga (Appendix 2.2). Unless stated otherwise, all results pertain to these dogs.

The sex ratio was approximately even in Johannesburg but skewed (male: female 75:25) in Bali through killing of unwanted female puppies (Appendices 2.3 and 2.4), and most dogs were adult (Appendix 2.5). The majority (>90%) of dogs were free to roam intermittently or continuously in Zenzele, Kelusa and Antiga, whereas in Braamfischerville approximately 40% of dogs were confined most of the time (Appendix 2.6). Most dogs were not sterilized except for 14.1% and 26.9% of male dogs in Kelusa and Antiga respectively. These dogs were “traditionally” castrated by a community member at about 6 months of age (Appendix 2.7).

2.3.2 Population size

Variations in population sizes are shown in Figure 2.1 and Appendix 2.8 (and age class at registration Appendix 2.9). Overall there was a decline in population size in Zenzele (linear regression $p<0.001$) and Antiga ($p<0.001$), while the population remained constant in Braamfischerville ($p=0.6$) and Kelusa ($p=0.5$), with no seasonal variation evident. The population decline in Antiga from March 2010 may be attributed to fewer dogs being gained during this period than prior to March 2010 (Mann-Whitney test $p=0.01$), whereas a similar number of dogs were lost during both periods ($p=0.5$) [the mean number of dogs gained per month before March 2010 was 12.4 and from March 2010 was 7.3; the mean number of dogs

lost before March 2010 was 10.6 and from March 2010 was 12.5]. Population size (and density) varied overall by a maximum of 22.1% (ranging from 3.8%) from the mean. The mean number of dogs gained and lost per month ranged between 10.3-18.7 and 11.4-20.3 respectively (Appendices 2.10 and 2.11). With the exception of Antiga, the percentage of dog-owning households was constant (Zenzele 12.0% at the start of the study and 10.0% at the end, Braamfischerville 7.5% and 7.8%, Kelusa 71.9% and 72.9%, and Antiga 49.0% declining to 41.6%) and the number of dogs per dog-owning household was unchanged at approximately 1.3 in Johannesburg and Antiga and 1.7 in Kelusa (Appendix 2.2).

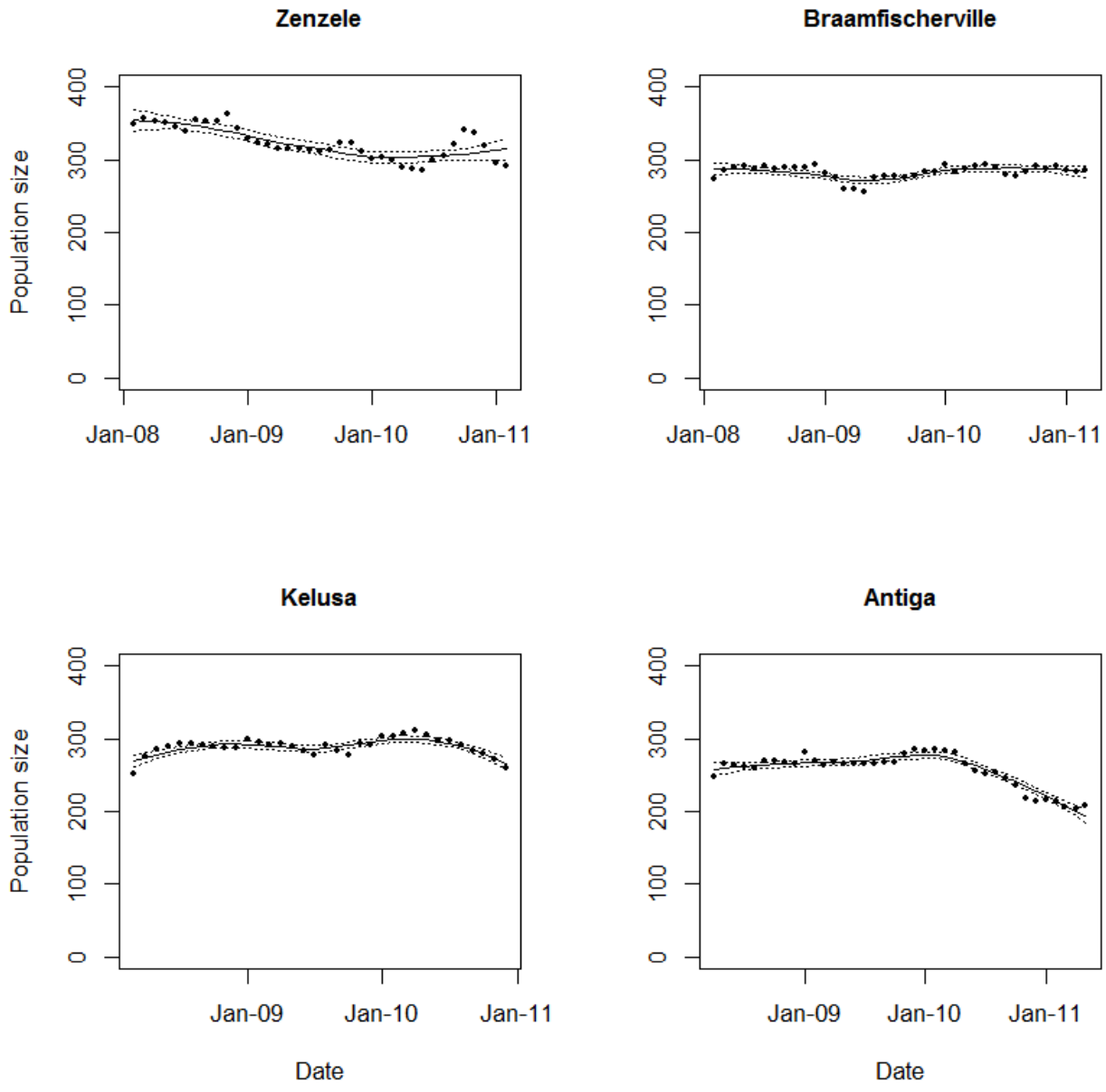

Figure 2.1 Variations in population size for the study period. Each large dot represents the number of dogs in the registered population at the end of that month after accounting for the dogs gained and lost during that month. The non-parametric regression line (-) shows the average variation in population size during the study period, and the small dotted lines (.) the 95% confidence intervals for the mean.

In Zenzele, Braamfischerville, Kelusa and Antiga 7.9%, 26.1%, 2.0% and 4.5% of the registered dogs respectively were reported as present during the previous inter-census or -revisit period by respondents but were not observed directly by the primary researcher and enumerators. Excluding the non-observed dogs from the data set for Zenzele, Kelusa and Antiga did not change the trends in population size. However, removal of non-observed dogs from Braamfischerville resulted in an overall increase in population size during 2008 and 2009 when the interval between surveys was intermittently longer than the other sites.

2.3.3 Demographic processes

There was no overall increase or decrease in the proportion of dogs pregnant and dying per month, and there was no seasonal variation (Appendices 2.12 - 2.16). In Kelusa total and "other" mortality for the entire study population increased significantly with time ($p<0.001$), and there was an overall increase in juvenile mortality ($p<0.01$). In Zenzele, juvenile mortality during the first inter-census period was lower than during the rest of the study period. Exclusion of this period (or even the first two months of the study) resulted in constant juvenile mortality ($p=0.2$).

At least one third of the population was sourced from outside the study area (Zenzele 40.8%, Braamfischerville 59.5%, Kelusa 36.5%, Antiga 43.0%) (Table 2.1). Owners reported a variety of reasons for obtaining a dog from outside the research site (see Methods and Materials), although the most common reason was opportunism (Appendices 2.17 and 2.18). Most owners had planned to get a dog (Appendix 2.19), including a proportion (Zenzele 56%, Braamfischerville 74%, Kelusa 16% and Antiga 11% minimum) of those who found their dog by chance outside the research site. There was no overall increase or decrease in the proportion of dogs acquired from outside the study area per month (Appendix 2.20). Less than one third of the dogs were born in the household, and 50% or less were born in the study area (Zenzele 50.2%, Braamfischerville 16.0%, Kelusa 37.8% and Antiga 31.3%) (Table 2.1). A substantial proportion (15-20%) of dogs disappeared, were stolen or unaccounted for (Table 2.2). Appendix 2.21 shows the outcomes of pups born in study households.

Table 2.1 Sources of the registered dogs.

	Zenzele	Braamfischerville	Kelusa	Antiga
sourced as pups				
born at address	128 (19.0%)	60 (9.9%)	128 (28.1%)	87 (24.5%)
elsewhere in study area	210 (31.2%)	37 (6.1%)	44 (9.7%)	24 (6.8%)
(address not reported)	(98)	(4)	(20)	(6)
non-study area of the research site	NA	12 (2.0%)	12 (2.6%)	10 (2.8%)
research site but area not known	NA	78 (12.8%)	73 (16.0%)	47 (13.2%)
outside research site	186 (27.6%)	221 (36.3%)	120 (26.4%)	112 (31.5%)
not known	25 (3.7%)	25 (4.1%)	28 (6.2%)	20 (5.6%)
sourced as juveniles or adults [a]				
non-study area of the research site	NA	5 (0.8%)	0	3 (0.8%)
inside study area	11 (1.6%)	0	0	0
research site but area not known	NA	21 (3.4%)	10 (2.2%)	7 (2.0%)
outside research site	89 (13.2%)	124 (20.4%)	34 (7.5%)	28 (7.9%)
not known	24 (3.6%)	26 (4.3%)	6 (1.3%)	17 (4.8%)
total acquired	673	609	455	355

[a] includes a small number of dogs where the age at acquisition was not reported but was most likely juvenile or adult (see Appendix 2.9)

Table 2.2 Outcomes of the registered dogs.

	Zenzele	Braamfischerville	Kelusa	Antiga
died	513 (70.2%)	355 (59.5%)	322 (72.0%)	264 (68.4%)
disappeared	58 (7.9%)	30 (5.0%)	58 (13.0%)	39 (10.1%)
stolen	23 (3.1%)	38 (6.4%)	0	0
given away in the non-study area of the research site	NA	13 (2.1%)	3 (0.7%)	4 (1.0%)
given away outside research site	40 (5.5%)	66 (11.1%)	14 (3.1%)	26 (6.7%)
given to meat trader	NA	NA	18 (4.0%)	14 (3.6%)
relocated outside research site with owner	39 (5.3%)	34 (5.7%)	11 (2.5%)	7 (1.8%)
dumped	0	0	0	5 (1.3%)
other	1 (0.1%)	0	0	2 (0.5%)
unaccounted for	44 (6.0%)	47 (7.9%)	17 (3.8%)	19 (4.9%)
given away in study area but not found by enumerators	13 (1.8%)	2 (0.3%)	0	0
given away in research site but area not known	NA	12 (2.0%)	4 (0.9%)	6 (1.6%)
total lost	731	597	447	386

2.3.4 Declines in the starting cohorts

At least 20% (Zenzele 28.2%, Braamfischerville 23.9%, Kelusa 39.6% and Antiga 51.7%) of the dogs registered during the starting censuses were present three years later at the end of the study (Figure 2.2). Assuming 60% vaccination coverage of the starting cohort, coverage was estimated to decline to between 22-33% after 24 months, and assuming an initial coverage of 80%, coverage would be expected to decline to between 29-43% after 24 months (Table 2.3). The yearly relative decrease of the starting cohorts varied across age classes, with greatest declines recorded in younger age classes (Table 2.4). In Bali, dogs that were adults at the start of the study remained in the starting cohorts on average significantly longer than those that were juveniles and pups ($p<0.001$). Similar trends were observed in Johannesburg (Appendices 2.22 and 2.23). Appendix 2.24 shows the outcomes of the dogs in the starting cohorts.

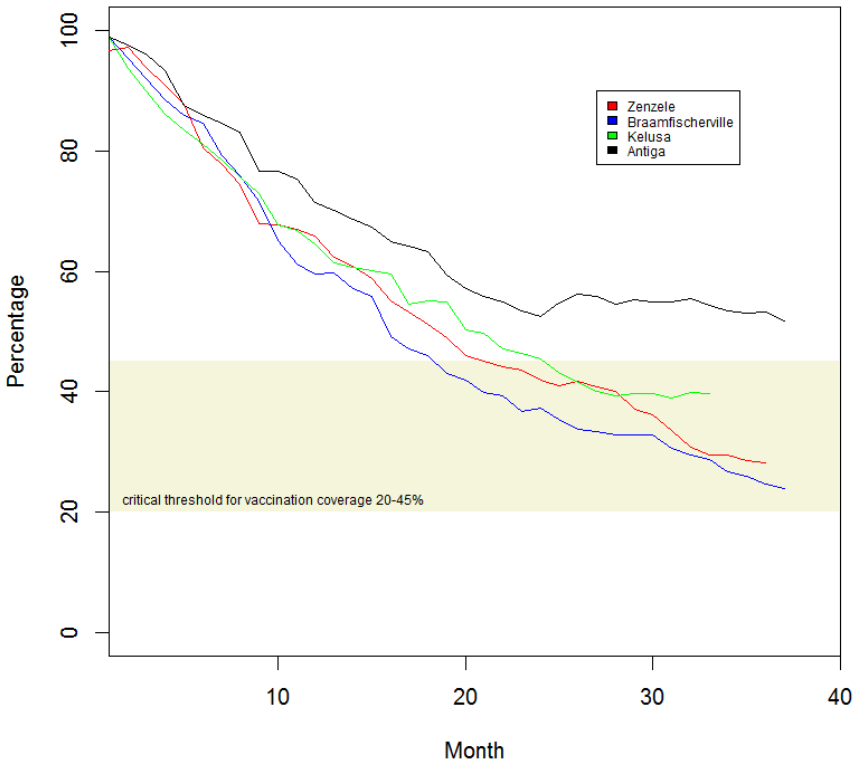

Figure 2.2 Declines in the starting cohorts for the study period, generated by plotting the number of dogs remaining from each starting cohort that month as a percentage of population size that month. The beige region indicates the vaccination coverage (of 20-45%) required to interrupt rabies transmission in a population (Hampson *et al.* 2009).

Table 2.3 Mean estimates of vaccination coverage with time (95% confidence intervals).

	% of the population vaccinated at month 0	% vaccination coverage after month 12	% vaccination coverage after month 24
Zenzele	60	40.0 (40.0-40.1)	26.1 (26.0-26.1)
	80	53.4 (53.4-53.5)	34.9 (34.9-35.0)
Braamfischerville	60	37.0 (36.9-37.1)	22.0 (21.9-22.1)
	80	49.3 (49.2-49.3)	29.2 (29.1-29.2)
Kelusa	60	40.2 (40.1-40.3)	27.4 (27.3-27.4)
	80	55.8 (55.8-55.9)	36.6 (36.5-36.7)
Antiga	60	43.2 (43.1-43.3)	32.6 (32.5-32.7)
	80	57.5 (57.4-57.6)	43.3 (43.2-43.4)

Table 2.4 Declines in number of dogs in the starting cohorts by age class and gender (males: females). The numbers of dogs are based on individual-level mid-point data which is a close approximation to the population-level averaged data.

	initial cohort of dogs	declines in cohort 0-12 months	declines in cohort 0-24 months	declines in cohort 0-36 months
Zenzele				
adults	276 (147:128)[c]	41.3% (65:48)	61.6% (90:79)	75.4% (109:98)
juveniles	73 (37:36)	43.8% (19:13)	76.7% (28:28)	84.9% (31:31)
pups	20 (14:6)	65.0% (7:6)	75.0% (8:7)	85.0% (10:7)
Braamfischerville				
adults	221 (113:107)[c]	41.6% (43:48)	60.6% (63:70)	72.9% (75:85)
juveniles	52 (33:19)	65.4% (22:12)	82.7% (27:16)	88.5% (30:16)
pups	15 (6:9)	53.3% (4:4)	66.7% (5:5)	86.7% (6:7)
Kelusa				
adults	209 (158:51)	27.3% (38:19)	44.5% (62:31)	58.4% (82:40)[a]
juveniles	43 (33:10)	55.8% (17:7)	60.5% (19:7)	72.1% (23:8)[a]
pups	27 (17:9)[c]	51.9% (10:3)	74.1% (13:6)	85.2% (13:9)[a]
Antiga				
adults	217 (172:43)[d]	24.4% (38:14)	38.2% (59:23)	53.9% (86:30)[b]
juveniles	32 (20:12)	43.8% (8:6)	68.8% (14:8)	81.3% (17:9)[b]
pups	19 (12:7)	52.6% (7:3)	73.7% (10:4)	89.5% (12:5)[b]

[a] 0-33 months; [b] 0-37 months; [c] the gender was not reported for 1 dog; [d] the gender was not reported for 2 dogs

2.3.5 Ownership

The participants of the Zenzele focus group agreed unanimously that all of the dogs residing in the study area were owned. Similarly, almost all of the dogs in Johannesburg identified by the primary researcher and enumerators were owned by households in the study areas. One healthy dog in Zenzele and one in Braamfischerville did not belong to a household in the study area (0.1% of all observed dogs in their third month of life or older in each study area); these dogs were each observed on only one occasion. One other dog owned by a household in Zenzele was abandoned. The dog was subsequently observed during successive survey periods around the rubbish heaps on the outskirts of the study area in worsening body condition (BCS 1-2), until it was eventually adopted by a neighbouring household.

Almost all of the dogs identified in the Bali sites were also owned by households in the study areas. Eight dogs in Kelusa and ten in Antiga did not belong to households in the study areas (1.1% and 1.5% of all observed dogs in their third month of life or older in each study area respectively). All of these dogs were observed on only one occasion, and almost all (12/16) were emaciated (BCS $\leq$2) and had severe generalised dermatitis (16/18). Although residents in the vicinity reported that at least six of these dogs were unowned, it was not verified if these dogs were owned by households outside the study area or were indeed unowned. In Kelusa 3.1% and in Antiga 5.3% of dogs owned by households in the study areas had BCS $\leq$2 and suffered from generalised dermatitis either consistently or transiently during the study period.

The majority of respondents in Bali (Kelusa 84% and 90% and Antiga 85% and 88% during the April 2008 and January 2009 censuses respectively) reported that there were no unowned dogs in either their banjar (April 2008) or village (January 2009). Most remaining respondents (71%) reported $\leq$10 unowned dogs in the banjar and village. These respondents assumed that dogs were unowned on the basis that they roamed (38%) and were often in poor condition or "uncared for" (48%) rather than known ownership status. Although limited to dog-owning respondents, given that most households have experience of owning a dog, these data may reflect community opinion overall.

2.3.6 Food sources

Almost all owned dogs had been reportedly fed by their owner on the day of the interview or the previous day. In Zenzele 2.6%, in Braamfischerville 0.6%, in Kelusa 0.1% and in Antiga 1.1% of dogs were last fed at least two days prior to the interview on one occasion and in Antiga 0.7% on more than one occasion. Only one juvenile in Antiga was reported as "never fed" by the owner, however long-term feeding patterns for this dog could not be established because it died two months after registration.

Most of the rubbish in the study areas consisted of inedible, inorganic matter. The remainder was poor quality organic matter, including food waste. Rubbish heaps around the periphery of Zenzele were removed by the community during the winters of 2009 and again 2010 after re-accumulation. The local authorities regularly collected household waste in Braamfischerville and very occasionally in Zenzele. In Kelusa and Antiga, organic matter was often incinerated or fed to pigs and occasionally used as compost. None of the sites were within 5 km of alternative food sources such as municipal rubbish dumps or commercial abattoirs.

Body condition scores were generally unimodal including individuals expected to have increased energy requirements from growth and lactation (Appendix 2.25). Although on average lactating individuals were thinner than non-lactating females and males, most were not underweight and body condition ranges were similar to non-lactating females and males. Overall, there was no tendency for growing individuals (i.e. pups and juveniles) to be thinner than adults (Appendices 2.26-2.30). Rather, on average young adults (13-36 months) had less subcutaneous fat than the other age classes probably consistent with normal anatomical variation (Lund *et al.* 2006). There was no interaction between age and lactation on body condition. Furthermore, even though sterilized dogs were on average fatter than unsterilized dogs, the relationship between body condition and age was similar for all four sites including Johannesburg where most dogs were not sterilized. There was no association between body condition and the number of dogs in the household.

2.4 Discussion

The longitudinal, individual-level data in this study provides the most detailed demographic data currently available for domestic dogs in low-income communities in Asia and Africa, and provides valuable support for planning disease and population control programmes and

parameterisation of epidemiological models of infectious diseases, including rabies, in these settings.

A key finding was that almost all of the identified dogs were owned by households in the study area, despite the vast majority being free-roaming, and were accessible for evaluation and vaccination. Our results are consistent with an interpretation that dog population size in these communities is regulated by human demand for dogs, not environmental resource constraints (i.e. food from refuse). We observed a range of body condition scores for these dogs regardless of energy requirements dependent on age or reproductive status, including lactation when energy requirements can be more than double (National Research Council 2006). This is consistent with owners reporting that most dogs were fed individually at the household on a regular basis. The low nutritional value of the refuse, and extremely poor body condition of most dogs not owned by households in the study area, also suggests that environmental resources were probably inadequate to meet the energy requirements of those dogs not fed properly by an owner, and that these dogs would not be able to survive in these environments without provisioning. Consistent with studies of environmental resource constraints in feral ungulate and wildlife populations (Clutton-Brock, Major & Guinness 1985; Coulson *et al.* 2001; Bonenfant *et al.* 2002), where mammalian population sizes fluctuate around K (Sibly & Hone 2002), if dogs are competing for environmental resources at the population level, then thin individuals may be those with the highest energy requirements. Although this association could be obscured by behaviours, such as hunting, domestic dogs are predominately scavengers (Bradshaw 2006) and, where refuse is the main environmental resource available to scavenge, such as in this study, this is unlikely. Thus, our data suggest that free-roaming, domestic dogs are not "wildlife", competing for environmental resources to survive; rather humans are responsible for providing adequate care for this domesticated species. Furthermore, while our observations were intermittent and limited to daylight hours, we did not observe a resident population of dogs in reasonable or good body condition that were not fed daily by an owner. Overall, these observations were consistent with community opinion expressed during this current study, and participatory exercises undertaken in Kelusa and Antiga during 2011 and 2012 that utilised systematic ranking methods to obtain consensuses regarding the food sources of free-roaming dogs (Chapter 3). While our findings may not be universally applicable, they agree with previous studies, primarily in sub-Saharan Africa, that report the majority of free-roaming dogs as owned and fed regularly by their owners (Brooks 1990; de Balogh, Wandeler & Meslin 1993; Butler & Bingham 2000).

Data from this study demonstrate the contribution of different demographic processes, including human-mediated movement, to variations in population size and support the view that dog population size is primarily a function of human factors. However, the cultural, economic and social factors driving the rates of acquisition and disposal of dogs (and thus ownership) are still poorly understood and warrant further investigation. This includes responses by community members to fear of rabies or liability arising from dog bites to people in rabies-endemic areas. Indeed a reluctance to acquire dogs because of rabies may have driven the declines in the Bali populations from January 2010 in the wake of a rabies epidemic. Population growth may, in part, be limited in established communities where geographical expansion is minimal and the number of household units relatively stable, such as in this study. Previous estimates of growth have generally been at the national (Brooks 1990) or district (Butler & Bingham 2000; Kitala *et al.* 2001; Hampson *et al.* 2009) level and may be reliable when considering the ecological heterogeneity within, and limited movement of dogs into and out of, large geographical areas. However, human-mediated movement of dogs, including over large geographical distances, can seed incursions of rabies and make endemic transmission more difficult to interrupt (Denduangboripant *et al.* 2005; Talbi *et al.* 2010; Townsend *et al.* 2013b). Therefore, the level or scale of interventions for control programmes and policy need to be considered carefully.

This study also has important implications for the design of vaccination campaigns, as the frequency of campaigns required to maintain vaccination coverage above the critical threshold of 20-45% (Hampson *et al.* 2009) depends on the introductions of susceptible individuals into the population by people through the acquisition of dogs born locally or from outside the population and the loss of vaccinated individuals through deaths and the relocation of dogs by people. We observed variable declines in the starting cohorts with time and age class. If 100% of these dogs had been vaccinated against rabies, and given the rates of birth, death and human-mediated movement recorded in this population, coverage sufficient to disrupt rabies transmission would have been maintained throughout the three year study period when using vaccine with a three-year duration of immunity. More realistically, vaccination coverage tend to fall in the range of 60-80%, with 80% achieved in the study areas during 2010, including the Bali sites where the dogs were less easy to handle. In these situations, vaccination coverage following a single campaign would decline to threshold levels after two years. Similar levels of vaccination coverage have been readily achieved in Africa (Kaare *et al.* 2009) and Asia (Bogel & Joshi 1990), and these results emphasise that the benefits of vaccination campaigns can be long-lived.

Our findings have practical consequences in terms of dog population control. Mass sterilization programmes, often used as an adjunct to vaccination and advocated as a necessary component of dog rabies control, will have a limited effect where population growth is limited and a large proportion of the population originates from outside the area. Furthermore, given the ongoing demand for dogs, any reduction in the local supply of puppies from sterilization, or following a cull, might result in further movement of dogs by people into communities from outside, compounding the risk of disease introduction.

2.5 Conclusion

Our results demonstrate the importance of human factors in the design and implementation of disease and population control programmes and epidemiological models. Although owners generally facilitate vaccination of their dogs against rabies, movement of dogs by people can increase the spread of rabies and, necessitating widespread and sustained vaccination programmes in rabies endemic areas. Human factors are therefore critical factors that must be considered in the development of disease and population control programmes.

Chapter 3

Participatory methods for the assessment of the ownership status of free-roaming dogs in Bali, Indonesia, for disease control and animal welfare

Summary

The existence of unowned, free-roaming dogs capable of maintaining adequate body condition without direct human oversight has serious implications for disease control and animal welfare, including reducing effective vaccination coverage against rabies through limiting access for vaccination, and absolving humans from the responsibility of providing adequate care for a domesticated species. Mark-recapture methods previously used to estimate the fraction of unowned dogs in free-roaming populations have limitations, particularly when most of the dogs are owned. We used participatory methods, described as Participatory Rural Appraisal (PRA), as a novel alternative to mark-recapture methods in two villages in Bali, Indonesia. PRA was implemented at the banjar (or sub-village) level to obtain consensus on the food sources of the free-roaming dogs. Specific methods included semi-structured discussion, visualisation tools and ranking. The PRA results agreed with the preceding household surveys and direct observations, designed to evaluate the same variables, and confirmed that a population of unowned, free-roaming dogs in sufficiently good condition to be sustained independently of direct human support was unlikely to exist.

3.1 Introduction

Understanding the characteristics of free-roaming dog populations is essential for the design of effective interventions to control canine diseases, such as rabies, and improve animal welfare. A critical issue relates to the possible existence of unowned, free-roaming dogs that are in sufficiently good condition to be sustained without direct human oversight. Ownership issues are critical for the design of rabies vaccination campaigns. Owners generally facilitate vaccination of their dogs against rabies (Lembo *et al.* 2010; Knobel *et al.* 2013), whereas unowned dogs are likely to be more difficult to identify and access for vaccination, potentially reducing effective vaccination coverage (Hampson *et al.* 2009), particularly if the fraction of unowned dogs is large. There is increasing evidence that most free-roaming dogs are owned and accessible for prophylaxis (Childs *et al.* 1998; Matter *et al.* 1998; Butler & Bingham 2000; Estrada *et al.* 2001; Kayali *et al.* 2003b; Windiyaningsih *et al.* 2004; Kaare *et al.* 2009; Lembo *et al.* 2010; Gsell *et al.* 2012; Putra *et al.* 2013). Previous studies, using mark-recapture techniques to evaluate vaccination coverage, generally indicate only a small

proportion (<10%) of free-roaming dogs are unowned in a range of urban and rural locations (Fishbein *et al.* 1992; Matter & Fico 1998; Matter *et al.* 1998; Cleaveland *et al.* 2003; Kayali *et al.* 2003b; Durr *et al.* 2009; Kaare *et al.* 2009; Gsell *et al.* 2012), although estimates with an upper confidence limit as high as 37% have been reported (Vos & Turan 1998; Matter *et al.* 2000; Kayali *et al.* 2003b). None of these studies reported the health status of unowned dogs, which remains an important gap in our understanding of these populations. However, there is a perception, implied by the implementation of interventions to reduce reproductive potential of unowned dogs, that these dogs are in sufficiently good condition for the population to be sustained without direct human oversight. An important corollary of this assumption is that it absolves humans from the responsibility of providing adequate care for a domesticated species.

During an intensive three-year study [April 2008 – December 2010] in the villages of Antiga and Kelusa, Bali, Indonesia, all identified, free-roaming dogs in the study area were monitored individually by direct observation and household questionnaire every 6-12 weeks (average of 250-300 dogs in each village) (Chapter 2). The study area encompassed most of the village and included every household in the main residential area. Almost all of the identified dogs were owned (i.e. belonged to a household in the study area) and fed regularly by their owner. Consistent with this finding was the observation that the vast majority of the owned dogs were in reasonable or good body condition, and only a small proportion (i.e. Antiga 5.3% and Kelusa 3.1%) "unhealthy" (i.e. with ribs clearly visible and concomitant generalised dermatitis). Only eight of the identified dogs in Kelusa and ten in Antiga did not belong to households in the study areas. All of these dogs were observed on only one occasion over the three year period, and almost all were emaciated (12/16) with severe generalised dermatitis (16/18). The poor condition of these dogs is consistent with the lack of edible refuse in the environment, based on subjective assessment, and householders reportedly rarely feeding dogs other than their own. Therefore, all of the healthy dogs resident in the study areas were identified as owned and fed by their owner, and there was no evidence for a resident population of dogs in reasonable or good body condition not fed daily by an owner.

Similarly, during household surveys the majority (~80%) of householders reported that there were no unowned dogs, with the remainder reporting generally ≤10 unowned dogs at any one time in the community. Householders generally assumed dogs to be unowned based on their health and confinement status (i.e. "thin with bad skin" and "on the street") rather than specific knowledge of an owner. Overall, these results suggested that a sub-group of

unowned dogs, in sufficiently good body condition to be sustained independently of direct human oversight, did not exist in these two villages. However, given the implications for rabies control and animal welfare, this study aimed to generate additional evidence relating to the ecology and health of free-roaming dog populations using an alternative approach, specifically community-level participatory exercises.

Community-based participatory methods, termed Participatory Rural Appraisal (PRA), have been used extensively for research purposes by those from outside the community (Chambers 1994a; Chambers 2007) including for veterinary epidemiology (Catley, Alders & Wood 2012) and rabies control (Okell *et al.* 2013). These methods facilitate the sharing of local knowledge, and typically involve visualisation tools and ranking or scoring, but may also include group discussion or semi-structured interviews (Chambers 1994a; Chambers 2007; Upjohn *et al.* 2013). Triangulation, or the comparison of PRA outputs with results generated by gold standard methods evaluating the same variables, is necessary to validate PRA outputs (Catley 1999; Catley, Alders & Wood 2012). Historically participatory approaches were developed to address discrepancies between perceived community-level issues determined through conventional surveys and by the community themselves (Catley 1999). While neither are gold standard methods, PRA outputs have been shown to agree with, and thus verify, key findings from a limited number of conventional surveys designed to assess the same variables (Chambers 1994b; Upjohn *et al.* 2013).

From previous studies (Putra *et al.* 2013), we assume that if a fraction of the free-roaming dog populations in Antiga and Kelusa was indeed unowned and in reasonable body condition, these individuals would comprise <10% of the population. Therefore, we preferred community-based participatory exercises to mark-recapture approaches given that it may be difficult to differentiate a real number of unowned dogs in reasonable body condition from measurement error and statistical variation, which may be large and encompass zero (Matter *et al.* 2000; Kayali *et al.* 2003b; Totton *et al.* 2010; Belsare & Gompper 2013). This may be compounded by violations of mark-recapture model assumptions, such as closed and stable populations. We determined *a priori* that population size was unlikely to remain constant between marking and recapture through frequent gains and losses of dogs (Chapter 2). Furthermore, the study populations were not closed and were confluent with the other populations in the non-study areas and neighbouring villages. Free-roaming dogs may travel substantial distances (Garde *et al.* 2012), therefore owned, unconfined dogs from the neighbouring villages may wander into the research villages.

We used PRA in Kelusa and Antiga, as a novel approach in dog ecology studies, to draw on local knowledge to obtain community-level consensus regarding the food sources of free-roaming dogs according to health and ownership status to infer the existence (or not) of unowned dogs in adequate body condition. Specifically, the PRA aimed to generate additional information about the health and ownership status of free-roaming dogs for triangulation of data from direct observations and household surveys.

3.2 Methods and materials

Participatory exercises were used to generate banjar-level discussion and consensus on the food sources of four independent categories of free-roaming dogs (owned, unowned, healthy and unhealthy). The exercises were designed to avoid associations between the categories so that ownership and health status were not confounded; thus, ownership status was not defined by health status.

Antiga included three banjars; and Kelusa six banjars, including one banjar not involved in the preceding surveys (Yehtengah). The banjar heads extended an open invitation to every person in their banjar to attend the PRA session. Sessions occurred from April 2011 to April 2012. With the exception of one session in Kelusa with female only participants, all sessions were held in the evening to maximise attendance. Sessions were mixed (male and female) for the Antiga banjars and for one banjar in Kelusa; however, because of cultural differences, sessions were divided into male and female for the remaining Kelusa banjars. Female sessions were run as part of the women's community groups due to a reluctance of women to attend mixed-gender banjar sessions. One banjar (Triwangsa) declined to host a female session, probably because of the caste divide unique to that banjar. The one banjar in Kelusa not involved in the preceding surveys was included in the PRA as a means to determine whether the preceding survey work may have confounded the PRA outcomes. Each session ran for approximately 3 hours and included a short video on rabies prevention at the end.

The participatory exercises were developed and implemented by a fully trained, experienced external facilitator from Praxis – Institute for Participatory Practices (India). Exercises were first developed and piloted with a team of seventeen Balinese who worked for three local animal welfare organizations including the one involved in the preceding surveys. All were trained to facilitate the planned PRA exercises as a team by the external facilitator during two sessions the week before starting in the banjars. A team was trained in anticipation of large numbers of participants for each PRA session.

The PRA sessions in the banjars were implemented by the Balinese team, with the external facilitator overseeing implementation by the team in Antiga. The external facilitator was not present in Kelusa. The exercises were carried out in Bahasa and Balinese, and all verbal and written outputs were recorded in English during the sessions. All outputs were drawn by the banjar participants to accommodate the less-literate; outputs were also written by literate participants as desired. Drawings were done on paper (A1 for body mapping and ~15cm^2 sheets for food source ranking) using coloured pens.

The exercises were in three sequential parts (i) semi-structured discussion at the banjar level, (ii) visualisation exercises at the group level with feedback at the banjar level, and (iii) ranking exercises at the banjar level (Appendix 3.1). The semi-structured discussion regarding dog ownership aimed to prepare the participants for the visualisation and ranking exercises and for open discussion throughout the session. The visualisation exercises involved group-level drawings of healthy and unhealthy dogs, followed by banjar-level discussion of the drawings to establish the body condition of healthy and unhealthy dogs. Finally, food sources for four independent categories of dogs – healthy, unhealthy, owned and unowned were discussed and ranked in order of importance at the banjar level. For each the participants were asked to ignore the other classification. For example, when ranking food sources for a healthy dog, participants ignored whether the dog was owned or unowned. Ranking was iterative, with rankings re-ordered based on discussion and debate, until consensus on the final rankings was reached. All possible food sources for each health or ownership category were listed. No attempt was made to quantify average volume of each food source in the diet of dogs in each category. With one exception (see Appendix 3.3), banjar attendees were divided into at least four groups for the group-level activities, with women in a separate group to ensure their involvement. The exercises were considered culturally appropriate given that Balinese are generally familiar with banjar-level meetings and are artistic.

A summary of the three highest ranked food sources for each health and ownership category and for each village are shown in Tables 3.1 and 3.2. The summary is derived from Appendices 3.5-3.8. For example, food purchased from a pet shop by the owner and food prepared by the owner for the dog were ranked as the most important food sources for healthy dogs in Kelusa. These two food sources are stipulated in Table 3.2 as rank 1 for healthy dogs.

3.3 Results

The sessions were well attended, generally ranging from approximately 80-110 participants per banjar in Antiga and 35-95 in Kelusa. Two male sessions in Kelusa were poorly attended - in Peliatan because of torrential rain and in Triwangsa because of the caste divide. In the mixed sessions, the majority of participants were male, with approximately 8-30 female participants and a small number of children or young teenagers (Appendix 3.2).

Contrary to the preceding household surveys, during the semi-structured discussions ownership was generally based on behaviour, e.g. with owned dogs identified through being responsive to a specific person, and skin condition, rather than body condition. Only one banjar (Ayah male group) differentiated owned from unowned dogs based on body condition.

At the group level, body condition was not consistently listed in relation to health status. However, with one exception, at the banjar level dogs with ribs clearly visible were deemed unhealthy (Appendices 3.3 and 3.4). At the group level the number of characteristics related to health ranged from 3-13 (mode 9) and were diverse, including skin condition, behaviour, reproductive health, appetite and body condition, and a range of clinical signs. At the banjar-level, dogs with a runny nose, watering eyes, lameness, ticks and bad skin were classified as unhealthy, except for lameness in Kaler (where opinion was split), runny nose in Roban, and eye discharge in Peliatan (where two participants disagreed with the consensus that these dogs were unhealthy).

Healthy and owned dogs had similar food sources, with food prepared by an owner ranked as the most important for all banjars. Unhealthy and unowned dogs had similar food sources, with rubbish and faeces ranked as the most important overall (Tables 3.1 and 3.2). Only two banjars (Kelikikawan and Roban) inferred that unhealthy dogs may be owned and probably neglected (Appendix 3.7). The results were similar for the banjar in Kelusa not included in the conventional surveys, and for male and female groups, although the women tended to suggest fewer food sources for owned and healthy dogs, restricted to food provided by an owner (Appendices 3.5 and 3.6).

Table 3.1 Summary of the three highest ranked food sources for Antiga (derived from Appendices 3.5-3.8 which show all the food sources and their ranks for each banjar).

status	rank	food source
healthy	1	prepared by owner / owner leftovers
	2	prepared by owner / offerings
	3	neighbour leftovers / rubbish
owned	1	prepared by owner / owner leftovers
	2	pig food / rubbish
	3	offerings / dead animals
unhealthy	1	rubbish / faeces / dead animals
	2	rubbish / dead animals
	3	rubbish / pig food
unowned	1	rubbish / faeces / pig food
	2	rubbish / pig food
	3	rubbish / offerings

Table 3.2 Summary of the three highest ranked food sources for Kelusa (derived from Appendices 3.5-3.8 which show all the food sources and their ranks for each banjar, including by participants' gender).

status	rank	food source
healthy	1	pet shop[a] / prepared by owner
	2	pet shop / prepared by owner / owner leftovers
	3	owner leftovers / stolen from the neighbour / stealing poultry
owned	1	pet shop / prepared by owner / owner leftovers
	2	pet shop / prepared by owner / owner leftovers / rubbish
	3	owner leftovers / stolen from the neighbour / rubbish
unhealthy	1	rubbish / faeces / dead animals / vermin
	2	rubbish / faeces / pig food / dead animals
	3	rubbish / faeces / pig food / dead animals / vermin
unowned	1	rubbish / faeces / stolen from householders / dead animals
	2	rubbish / faeces / pig food / offerings / dead animals
	3	rubbish /pig food / offerings / stealing poultry / vermin

[a] the purchase of pet food by owners in Kelusa, but not in Antiga, is consistent with the socioeconomic differences between the villages

3.4 Discussion

This study demonstrates the benefit of drawing on local knowledge through community-based approaches in dog ecology studies. However, our research also highlights the challenge of definitively identifying resident unowned, healthy dogs, particularly where most of the dogs are owned.

The results from this study were consistent with the preceding household surveys and direct observations which suggested that a resident population of healthy, unowned dogs was unlikely to exist. It had been determined *a priori* by these household surveys and direct observations that a. all of the healthy dogs resident in the study areas belonged to households and were fed regularly by the householders, b. a minority of dogs belonging to households in the study areas were underweight, c. the majority of the dogs not belonging to a household in the study area were emaciated (although residents in the study area reported that at least six of these dogs were unowned, it was not verified if these dogs were actually owned outside the study areas) and, d. a resident population of dogs in reasonable or good body condition not fed daily by an owner was not apparent. This suggests that healthy dogs were owned and fed by their owners and, consistent with an apparent lack of edible refuse in the environment, dogs not fed adequately by an owner were unable to find sufficient environmental resources to meet their energy requirements.

These *a priori* observations are supported by the PRA results. Firstly, there was no perception at the banjar level that the most important food source for healthy dogs was anything other than an owner. Rather, food from an owner was ranked as the most important food source for healthy and owned dogs. Secondly, similar food sources were listed for unowned and unhealthy dogs, suggesting that unowned dogs are indeed unhealthy. The food sources ranked as most important for these dogs included rubbish, faeces and dead animals. Consistent with *a priori* observations, these food sources probably provide insufficient nutrition for free-roaming dogs and, therefore, unowned dogs are unhealthy through poor nutrition. Taken together, these results imply that it was unlikely that a resident population of unowned, free-roaming dogs in reasonable or good body condition, existed in Kelusa and Antiga that were effectively "invisible" amongst the owned, free-roaming dogs. A key implication of this finding is that, to maintain a reasonable health status, dogs are dependent upon direct provisioning by people; free-roaming dogs should not be considered as "feral" populations and people cannot be absolved from the responsibility of providing adequate care for this

species. Results from this study indicate that almost all of the dogs in Kelusa and Antiga are owned and are, therefore, likely to be accessible for vaccination.

Although the key findings from the PRA and direct observations and surveys were consistent and similar to previous studies (Chambers 1994b; Upjohn *et al.* 2013), there were also important discrepancies. A high ranking score indicated that some food sources other than from the owner were perceived to be important (Tables 3.1 and 3.2 and Appendix 3.6), which did not accord with the preceding direct observations or survey results. In these surveys generally <2% of dogs were reported to have eaten rubbish or other food outside the household, and owned dogs were infrequently observed scavenging (Chapter 2). The discrepancy may be attributable to the PRA methodology with the participants encouraged to "brain storm" all possible food sources for each category of dog, and no attempt was made to determine the volume or frequency eaten of a particular food type. It may also reflect the contrast between the mixed and male groups and the women's groups. The women generally restricted food sources for healthy and owned dogs to the owner (Appendices 3.5 and 3.6). These results are more consistent with the preceding survey results and may be more reliable given that generally the women organise the food and feed the dogs in this society.

This study was designed to identify the existence of a resident population of unowned, healthy dogs, if there was one, by inference from local knowledge. An important consideration is that, for ecological studies such as this, PRA is limited to verification of data collected by conventional methods and is not optimal when used as a sole modality. For example, had rubbish been consistently ranked equal to or higher than an owner for healthy dogs then, from this result alone, it would not be possible to differentiate between a. a resident population of genuinely unowned healthy dogs in the study area, b. some or all of the owned dogs in the study area obtaining a proportion of their nutritional requirements from rubbish, or c. the community misidentifying dogs owned outside the study area and that wander into the study area from unowned dogs. However, owner derived food was consistently associated with both health and ownership; this does not directly address the question whether unowned and healthy dogs existed, but failed to provide any evidence for their existence in any banjar. This is also supported by the observation that nutritional sources for unowned and unhealthy dogs were of poor quality and the same as those that would have been available to unowned, healthy dogs had they existed. Had PRA results diverged from results generated by our conventional methods, or had PRA been used as a sole modality, then additional objective methods would have been invaluable to triangulate the PRA results. For the example above, this would involve approaches that might have included further,

intensive focus groups, or more technically demanding methods such as monitoring the movement of dogs with GPS collars.

3.5 Conclusion

This study has demonstrated the value of alternative approaches to mark-recapture to establish the presence of unowned dogs in adequate body condition in free-roaming populations, particularly where the fraction is expected to be small. While questionnaires have been used previously for this purpose (Butler & Bingham 2000), PRA is a novel approach that can generate additional data to complement conventional surveys designed to evaluate the same variables. This study provides further evidence that there is unlikely to be a population of free-roaming dogs in Bali that is capable of maintaining adequate health without any direct human oversight, with fundamental implications for disease control and animal welfare.

Chapter 4

Achieving population-level immunity to rabies in free-roaming dogs in Africa and Asia

Summary

Canine rabies can be effectively controlled by vaccination with readily available, high-quality vaccines. These vaccines should provide protection from challenge in healthy dogs for the claimed period for duration of immunity, which is often two or three years. It has been suggested that, in free-roaming dog populations where rabies is endemic, vaccine-induced protection may be compromised by immuno-suppression through malnutrition, infection and other stressors. This may reduce the proportion of dogs that seroconvert to the vaccine during vaccination campaigns and the duration of immunity of those dogs that seroconvert. Vaccination coverage may also be limited through insufficient vaccine delivery during vaccination campaigns and the loss of vaccinated individuals from populations through demographic processes. This is the first longitudinal study to evaluate temporal variations in rabies vaccine-induced serological responses, and factors associated with these variations, at the individual level in previously unvaccinated free-roaming dog populations. Individual-level serological and health-based data were collected from three cohorts of dogs in regions where rabies is endemic, one in South Africa and two in Indonesia. We found that the vast majority of dogs seroconverted to the vaccine; however there was considerable variation in titres, partly attributable to illness and lactation at the time of vaccination. Furthermore, >70% of the dogs were vaccinated through community engagement and door-to-door vaccine delivery, even in Indonesia where the majority of the dogs needed to be caught by net on successive occasions for repeat blood sampling and vaccination. This demonstrates the feasibility of achieving population-level immunity in free-roaming dog populations in rabies-endemic regions. However, attrition of immune individuals through demographic processes and waning immunity necessitates repeat vaccination of populations within at least two years to ensure communities are protected from rabies. These findings support annual mass vaccination campaigns as the most effective means to control canine rabies.

4.1 Introduction

Canine-mediated rabies is a viral zoonosis, causing at least 55,000 human deaths every year (Knobel *et al.* 2005). Mortality from rabies is highest in less developed communities in Asia and Africa, where domestic dogs are free-roaming (Ezeokoli & Umoh 1987; Butler &

Bingham 2000; Kitala *et al.* 2002; Kayali *et al.* 2003a; Windiyaningsih *et al.* 2004; Reece & Chawla 2006; Kasempimolporn, Jitapunkul & Sitprija 2008); with increasing evidence that the majority are owned (Ezeokoli & Umoh 1987; Butler & Bingham 2000; Estrada *et al.* 2001; Windiyaningsih *et al.* 2004; Gsell *et al.* 2012) and, thus, generally accessible for vaccination (Lembo *et al.* 2010; Knobel *et al.* 2013).

Canine rabies can be effectively controlled by vaccination (Cleaveland *et al.* 2003; Schneider *et al.* 2005; Cleaveland *et al.* 2006; WHO 2013) using readily available, high potency (antigenic value ≥1 IU/ml), inactivated cell-culture vaccines. These vaccines should provide protection from challenge in healthy dogs for the claimed period for duration of immunity (Council of Europe 2008), which is often two or three years. In free-roaming dog populations, vaccine-induced protection from rabies may be compromised for several reasons. These include: (a) insufficient vaccine delivery during vaccination campaigns (Lembo *et al.* 2010), (b) lack of repeat vaccination campaigns, with loss of vaccinated individuals from populations through demographic processes (Jackson 2013) (Chapter 2), and a substantial proportion of dogs probably vaccinated only once in their lifetime (Mitmoonpitak, Tepsumethanon & Wilde 1998), despite them often living beyond three years of age (Chapter 2); and, (c) the possibility of immuno-suppression through malnutrition, infection or other stressors (MSD Animal Health; Dionigi *et al.* 1977; Roitt, Brostoff & Male 2001), which may reduce the proportion of dogs that seroconvert or the duration of immunity of those dogs that seroconvert. These constraints may result in a decline in the vaccination coverage between campaigns to below 20-45%, the threshold necessary to control rabies (Hampson *et al.* 2009). Consequently, investigating the effectiveness of vaccination campaigns under field conditions is critical.

The adaptive (B-cell humoral and T-cell cell-mediated) immune response to vaccination is complex. The humoral response generates virus neutralizing antibody (VNA), the primary correlate of protection induced by viral vaccines (Siegrist 2008; Johnson, Cunningham & Fooks 2010; Moore & Hanlon 2010; Jackson 2013). Cell mediated immunity (CMI) is also important for the development of vaccine-induced immunity (Thraenhart *et al.* 1994; Arya & Agarawal 2006; Corradi, Ferrari & Borghetti 2007) and acts in synergy with the humoral response (Siegrist 2008). Ongoing protection from challenge depends on the persistence of long-lived plasma cells, continuing to generate antigen-specific antibody, and B- and T-memory cells. The primary antibody response following vaccination generally correlates with the strength of the memory response (B- and T-cell) and, thus, the ability to induce secondary responses to subsequent challenge (Sikes *et al.* 1971; Brown, Merry & Beckenhauer 1973;

Bahloul *et al.* 2006; Lodmell *et al.* 2006; Hu *et al.* 2008; Siegrist 2008). In healthy dogs the quality of the primary immune response to vaccination depends on several factors, including the type of vaccine, with modified-live vaccines generally inducing superior responses, the route of administration, and the dose of vaccine antigen (Brown, Merry & Beckenhauer 1973; Aubert 1992; Lodmell *et al.* 2006; Hu *et al.* 2008; Siegrist 2008; Minke *et al.* 2009; Johnson, Cunningham & Fooks 2010).

Laboratory challenge studies in healthy dogs support these observations. Following seroconversion, protection from rabies virus challenge correlates with peak VNA titre and final titre prior to challenge for inactivated, DNA and modified-live vaccines, with increased susceptibility to challenge once titres drop to near negligible levels (VNA titres <0.1 IU/ml or mouse serum neutralizing antibody titres <1:2 dilution) (Sikes *et al.* 1971; Brown, Merry & Beckenhauer 1973; Bunn, Ridpath & Beard 1984; Precausta *et al.* 1985; Sharpee, Nelson & Beckenhauer 1985; Bunn 1991; Aubert 1992; Bahloul *et al.* 2006; Lodmell *et al.* 2006; Hu *et al.* 2008). These studies used comparable antibody assays (Cliquet, Aubert & Sagne 1998; Ondrejkova *et al.* 2002) and virus challenge doses. Titres measured repeatedly over 3-4 years initially peaked and then declined rapidly, followed by a more gradual decline (Sikes *et al.* 1971; Brown, Merry & Beckenhauer 1973; Sharpee, Nelson & Beckenhauer 1985; Bahloul *et al.* 2006). While a titre of 0.5 IU/ml demonstrates seroconversion following vaccination (Kennedy 1998), the approximate threshold for protection following seroconversion may be 0.1 IU/ml (Precausta *et al.* 1985; Aubert 1992; Bahloul *et al.* 2006; CDC 2008). However, in the aforementioned experimental studies, only a proportion (<40%) of dogs with measureable titres following vaccination, but with negligible titres at the time of challenge succumbed to challenge, highlighting the importance of previously activated B- or T- cells allowing rapid response to challenge.

Although the same relationship between VNA titre and protection from challenge is expected in immuno-suppressed dogs as in healthy dogs (MSD Animal Health; Roitt, Brostoff & Male 2001), no systematic comparison has been published to date. Reduced humoral immune responses have been shown in malnourished experimental dogs (Dionigi *et al.* 1977) and Gambian children vaccinated with human diploid-cell rabies vaccine (Moore *et al.* 2003), and pet dogs with anaemia or intestinal parasites vaccinated against rabies (Tepsumethanon *et al.* 1991; Aubert 1992). Several studies have evaluated the immune response in previously unvaccinated, mostly healthy pet dogs to high potency, inactivated rabies vaccine under field conditions (Tepsumethanon *et al.* 1991; Wilde *et al.* 1991; Sage *et al.* 1993; Sihvonen *et al.* 1996; Cliquet *et al.* 2003; Jakel *et al.* 2008; Berndtsson *et al.* 2011). All of these studies

report variable VNA titres up to 12 months following vaccination, including a proportion of dogs with titres ≤0.1 IU/ml (and generally a larger [17% to >42%] proportion with titres <0.5 IU/ml). These observations have serious implications for free-roaming dogs where their health status is more likely to be compromised. However, with the exception of one study in Peru (Chomel *et al.* 1987), no study has evaluated variations in vaccine-induced VNA in previously unvaccinated free-roaming dogs where rabies is endemic. Furthermore, no study has properly evaluated the factors associated with these variations.

Cell-mediated immunity is technically difficult to measure under field conditions (Tizard & Ni 1998; Corradi, Ferrari & Borghetti 2007), however peripheral blood lymphocyte counts, which are predominately T-cells (Weiss & Wardrop 2010), may provide a straightforward, indirect assessment of CMI. Together with cytokine assays and measures of blastogenic responses of lymphocytes to mitogen, lymphocyte counts were used to assess immunomodulation in healthy dogs in response to vaccination (Phillips *et al.* 1989; Miyamoto *et al.* 1992; Strasser *et al.* 2003) and protein-calorie malnutrition (Dionigi *et al.* 1977), and in humans in response to protein-calorie malnutrition (Baron 1986). In dogs, malnutrition induced declines in immunoglobulin and lymphocyte function and counts. Therefore, lymphocyte counts together with rabies vaccine-induced titres and nutritional status may correspond to the overall immune status of an individual and susceptibility to infection.

This study focused on evaluating temporal variations in vaccine-induced VNA, and factors associated with these variations, in three previously unvaccinated, owned free-roaming dog populations in South Africa and Indonesia, to better understand their effect on vaccination coverage. In addition, the efficiency of vaccine delivery and loss of vaccinated individuals from the cohorts were also assessed.

4.2 Methods and materials

4.2.1 Study populations

See Appendix 4.1 for a summary of the methodology. Data were collected from three cohorts of dogs, one in South Africa, and two in Indonesia. The cohorts were part of a larger ecological study that commenced in March 2008 (Chapter 2). The South African cohort was located in Zenzele, an informal settlement 10 km west of Johannesburg (26.15^{o}S and 27.41^{O}E). In Indonesia the cohorts were located in the study areas of Kelusa (8.26^{o}S and 115.15^{o}E) and Antiga (8.30^{o}S and 115.29^{o}E), two villages on the island of Bali. Kelusa,

composed of six banjars (sub-villages), is inland. The study area encompassed the entire village except for Banjar Yehtengeh, separated from the rest of the village by rice fields and jungle, the southern half of Banjar Kelikikawan and the households scattered along the main road leading into the village. Antiga, a large village of six banjars, is located on the east coast. The bulk of the households are clustered into two banjars (Kaler and Kelod). The study area encompassed all of Kaler and Kelod. An additional area (Banjar Ketug) included households scattered along a 2.7 km stretch of road winding through the jungle north of Kaler and Kelod. Rabies is endemic in Indonesia and South Africa, with outbreaks occurring in Bali in 2008 and Gauteng Province in 2010.

The Zenzele research cohort included every available dog in the entire township (which was the study area) in February 2010 that had not been previously vaccinated by the Department of Agriculture (DoA) during a vaccination point (VP) on the outskirts of the township in October 2009 (Appendix 4.2). All the dogs vaccinated by the DoA were identified within one week of the one day VP through a rapid door-to-door search, with verification by owners and inspection of certificates. The DoA had also set-up a VP on the outskirts of Zenzele in May 2006, thus vaccination history and certification were checked with each owner at the start of the study. VNA titres were also evaluated for anamnestic responses to vaccination consistent with previous vaccination.

The Bali research cohorts included every available dog in the study areas of Kelusa and Antiga in January 2010 that had not been previously vaccinated by the Department of Livestock (DoL) as described below (Appendix 4.2). Prior to a rabies outbreak in 2008, vaccination against rabies was illegal in Bali and there had been no systematic vaccination programmes in either village prior to commencement of the study. Vaccination points were set up by the DoL in two banjars in Kelusa in December 2009 and in one banjar outside of the study area in Antiga in February 2010. The VPs were poorly attended because of community awareness of the research vaccination programme and because the owners could not readily handle their dogs. In Kelusa, 16 dogs from the study area attended the vaccination points. In Antiga only three dogs from the study area attended the vaccination point.

All of the dogs resident in the study area were owned and had been previously identified by household, name and appearance through intensive monitoring by direct observation and survey since March 2008. Intensive monitoring of all of the dogs in the study area continued until April 2011. Therefore, all of the dogs in the study population were readily identified at the individual level during the study period. There was no evidence for a resident population

of unowned dogs (Chapters 2 and 3). All dogs in their third month of life or older were photographed (standardised dorsal and lateral views). Pups in their first or second month of life were recorded but not photographed. The same enumerators had tracked the majority of the cohorts at the individual level since March 2008 and were familiar with the dogs.

4.2.2 Vaccination and sampling

Vaccine delivery was door-to-door for the research cohorts, and households were revisited repeatedly until the dog was caught for vaccination and blood sampling, or it was apparent that the dog could not be caught or the owner would not be available to give consent. A dog was also excluded from the study if the owner declined consent, the dog did not remain calm during restraint, there was a high index of suspicion that the dog may bite, or it was apparent the dog had a clinical condition that might have deteriorated as a result of restraint.

All the dogs were carefully restrained by experienced personnel using the correct equipment and under the direct supervision of a veterinarian. In Zenzele, dogs were gently restrained with a leash and soft muzzle. In Bali most dogs could not be safely restrained by leash and muzzle and required restraint by net. Vaccinations and blood sampling were undertaken by experienced veterinarians. High-quality, sterile consumables (i.e. needle, syringe and blood tubes) were used for each vaccination and blood sample. Dogs in the research cohorts were vaccinated with 1ml of Rabisin (Merial Animal Health Limited), an inactivated rabies vaccine containing at least 1 IU/ml of rabies virus glycoprotein (GS57 Wistar strain) with an aluminium hydroxide adjuvant. Vaccine was administered subcutaneously into the neck or shoulder region. The vaccine cold chain was carefully preserved.

Rabisin and Galaxy DA2PPv, a polyvalent vaccine against common infectious pathogens, was administered by the DoA during the October 2009 VP in Zenzele. Some dogs vaccinated at the VP may have received ivermectin. The DoL administered Rabisin during the February 2010 VP in Antiga, and Rabivet Supra 92, a locally produced cell-culture vaccine, during the December 2009 VP in Kelusa. Vaccine administration and storage by the local authorities were not observed.

Different blood sampling schedules were required for Zenzele and Bali given the different methods of restraint and because the rabies outbreak in Bali escalated during 2009, forcing vaccination to be undertaken 6 months earlier than planned. Every dog in each research cohort, including neonates, was vaccinated at the start of the study (day 0) (Zenzele n=259, Kelusa n=284 and Antiga n=259 vaccinated [Appendix 4.2]), and every available dog from

about 6-8 weeks of age was blood sampled (see Table 4.1 and Appendix 4.3 for the number of dogs blood sampled at each time point).

Blood was collected from the Zenzele research cohort on day 0 (immediately prior to vaccination) and then approximately 30, 90, 180 and 360 days following vaccination. The dogs vaccinated by the DoA were also blood sampled 8-10 days after the VP. Samples were then collected approximately 30, 90, 180 and 360 days following the VP. In Zenzele, only those dogs that had been vaccinated were blood sampled. Rabies vaccine-induced VNA was measured at each time point. Complete blood counts (CBCs) measured on days 0, 180 and 360 for the research cohort.

In Bali, samples were collected on approximately day 180 and 360 following vaccination. Every available dog, whether vaccinated or not, was blood sampled at both time points and analysed for rabies vaccine-induced VNA. Unvaccinated dogs constituted the control group, and included those dogs not caught for vaccination on day 0 and those that arrived into the study populations after day 0. The sixteen dogs in Kelusa and three dogs in Antiga vaccinated by the DoL, in December 2009 and February 2010 respectively, were blood sampled at the same time as the research cohort.

In all the sites, households were visited in approximately the same order at each time point, so the number of days between samples were similar for each dog.

Table 4.1 The number of dogs in the research cohorts and the number of unvaccinated controls in Bali that were blood sampled at each time point (this table is reproduced with additional information in Appendix 4.3).

	day 0[a]	day 30	day 90	day 180	day 360
Zenzele vaccinated dogs	190	183	148	134	103
Kelusa vaccinated dogs	–	–	–	168	124
Kelusa unvaccinated dogs	–	–	–	70	79
Antiga vaccinated dogs	–	–	–	163	126
Antiga unvaccinated dogs	–	–	–	35	49

[a] day 0 immediately prior to vaccination for the research cohort

For each sample, 5-7ml of blood was collected from the jugular or cephalic vein and divided into plain and ethylene diamine-tetraacetic acid (EDTA) containing blood tubes. The blood tubes were immediately coded by date, house number and dog identification and placed in cool boxes with ice packs. Serum was separated by centrifugation within 8 hours of collection and refrigerated at 4-6^{o}C for up to 48 hours prior to freezing. All the sera were transported frozen in dry shippers to the Weybridge Animal Health Veterinary Laboratory Agency in the United Kingdom for fluorescent antibody virus neutralization (FAVN) assays. EDTA whole blood samples were refrigerated and then tested within 48 hours of collection for CBCs. Approximately 10 grams of faeces was collected manually on day 0 from 107 dogs randomly selected from the Zenzele cohort for routine analysis. Upon collection, the faecal sample pots were similarly coded and kept in the cool boxes, then refrigerated until being tested. Complete blood counts and faecal analysis were undertaken by the Faculty of Veterinary Science, University of Pretoria. Suitable laboratory facilities were not accessible in Bali for these tests. Finally, 32 dogs from Kelusa and Antiga combined were selected on day 180 from those dogs diagnosed with generalised dermatitis during the preceding survey for deep skin scrapes (DSS) from affected areas of skin to determine the prevalence of *Demodex spp*. See Appendix 4.4 for an explanation of sample selection for the DSS and faecal analysis.

4.2.3 Covariates

Factors that may influence the immune response to rabies vaccine were selected on their measurability under field conditions, particularly by vaccinators. These factors had been previously quantified at the individual level as part of the larger ecological study that commenced in March 2008, and the methods used to quantify the factors are described elsewhere (Chapter 2). In summary, the factors were categorical and measured by direct observation and questionnaire at the time of vaccination (gender, age class , pregnancy, lactation, sterilization status [Bali only], intestinal parasites [Zenzele only]) or within 6 weeks of vaccination (body condition, clinical signs associated with serious illness, protein intake [Bali only], and generalised dermatitis [Bali only]) (Dionigi *et al.* 1977; Tepsumethanon *et al.* 1991; Aubert 1992; Roitt, Brostoff & Male 2001; Moore *et al.* 2003; Mansfield *et al.* 2004; Kennedy *et al.* 2007; Guaguere, Prelaud & Craig 2008; Miller, Griffin & Campbell 2013). See Appendix 4.18 for a detailed description of the covariates. Time (points) was treated as a continuous variable.

4.2.4 Analytical methods

4.2.4.1 Laboratory tests

VNA was measured by fluorescent antibody virus neutralization (FAVN), a method prescribed by the Office International des Epizooties (OIE) (Cliquet, Aubert & Sagne 1998). In order to evaluate the variability in titres, including ≤0.1 IU/ml, the assay was modified to include a two-fold dilution with reciprocal dilutions ranging from 2 to 4096. Fifty percent endpoint titres, estimated by the Spearman-Karber method (WHO 1996), were converted into international units (IU/ml) by comparison with a standard serum. All samples were tested within two weeks of thawing and re-frozen within three weeks of testing. Except during assay preparation, all thawed samples were refrigerated.

All of the samples from the same dog were tested within the same batch. Consequently, samples from each dog were frozen for a variable amount of time between collection and testing and a proportion of the samples were stored for over 12 months. To evaluate the effect of storage time and freeze-thaw cycles on titres, 25 samples were randomly selected from the first batch tested. These samples had been frozen (-20^{o}C) for over 2 years between the initial and repeat tests.

To rule out cross-reaction with Lyssaviruses other than Rabies Virus (RABV), 30 samples were randomly selected from the Zenzele research cohort (day 0) and 60 from the Bali research cohorts and controls (day 180 and 360) combined and tested against Lagos Bat Virus (LBV), an antigenically divergent virus from Phylogroup II Lyssaviruses (Horton *et al.* 2010).

Complete blood counts were determined by an automated cell counter (ADVIA 2120 Siemens) using impedence counting, flow cytochemistry, laser light scattering and validated vet package software. The differential leukocyte counts were confirmed by manual counting.

Deep skin scrapes and faecal samples were evaluated using standard protocols (Soulsby 1982; Miller, Griffin & Campbell 2013) (Appendix 4.4).

4.2.4.2 Statistical methods

A range of models were used to explore the relationship between time after vaccination and physiological and health status at the time of vaccination on titre. Correlation coefficients for titres and log titres were determined for a combination of time points (i.e. day 30, 90, 180 and

360) for the vaccinated dogs in Zenzele. This suggested that dogs with higher peak VNA titres also had higher titres towards the end of the study period (further described in Results section 4.3.4.2).

Linear mixed effects models were fitted to the longitudinal data from the vaccinated dogs in the research cohorts using the nlme package in R (3.0.1) (Pinheiro *et al.* 2013; R Core Team 2014). Dogs vaccinated by the local authorities in Zenzele in October 2009, Kelusa in December 2009 and Antiga in February 2010 were excluded from these analysis because the administration of a standardised dose of Rabisin was not observed. The response variable, of VNA titre (here after referred to as "titre") following vaccination, was modelled as the natural log of the titre (determined by Box-Cox transformation) expressed in IU. Therefore, baseline (day 0) titres were dropped from the Zenzele models and the unvaccinated (control) dogs were excluded from the Bali models. Explanatory variables included time (points) and the covariates described in section 4.2.3 (and Appendix 4.18) as fixed effects, and dog as a random effect. All individuals with complete information for the variables of interest were included in the models. Forward and backwards stepwise regression compared the full range of covariates and their biologically plausible interactions to the null model. The models with the lowest Akaike's Information Criteria (AIC) for the highest number of observations were retained.

Models were first fitted to each cohort separately. The Bali cohorts were then combined and the model refitted with dog nested within study area (i.e. village) as a random effect. Finally, all the research cohorts were combined and the models refitted. Each of these models were fitted with and without upper outliers (i.e. day 30 titres ≥128 IU/ml for 7 dogs in Zenzele, and day 180 titres ≥11.3 IU/ml for 4 dogs in Kelusa and 15 dogs Antiga) in order to exclude dogs from the analysis that may have been previously vaccinated by the DoA in Zenzele in May 2006, as part of vaccination campaigns outside of Kelusa and Antiga, or privately by their owners. Upper outliers were defined according to vaccination history, breed, age, source, geographical location and post-vaccinal titres (further described in Results section 4.3.3).

The models take the form:

$$\ln\left(Y_{ij}\right) = \beta_0 + \beta_1 X_{ij1} + \cdots + \beta_p X_{ijp} + \beta_{p+1} X_{ijp}^2 + \theta_i + \varepsilon_{ij},$$

where Y_{ij} is titre and X_{ijk} ($k = 1, \ldots, p$) are the covariates for observation $j = 1, \ldots, n_i$ on individual $i = 1, \ldots, m$, where the final covariate (X_{ijp}) is time. Hence, time is modelled as a

quadratic curve (Figure 4.1). The vector $\beta = (\beta_0, \ldots, \beta_{p+1})$ is a vector of regression coefficients, and the vector $\theta = (\theta_1, \ldots, \theta_m)$ corresponds to a set of individual-level random effect terms, such that $\sum_{i=1}^{m} \theta_i = 0$. Finally the error terms $\varepsilon_{ij} \sim N(0, \sigma^2)$.

This model was fitted to the full data set for Zenzele, which included all the time points (i.e. day 30, 90, 180 and 360). The data set included one to four data points for each individual depending on the availability of the individual for blood sampling during the study period. Times were adjusted by 30 days to allow the model intercepts to correspond to day 30 (peak) titres. A model using an exponential decay (rather than quadratic) over time was also fitted, however the quadratic model provided a marginally better fit to the data across this range, and so only the results from the quadratic model are reported here.

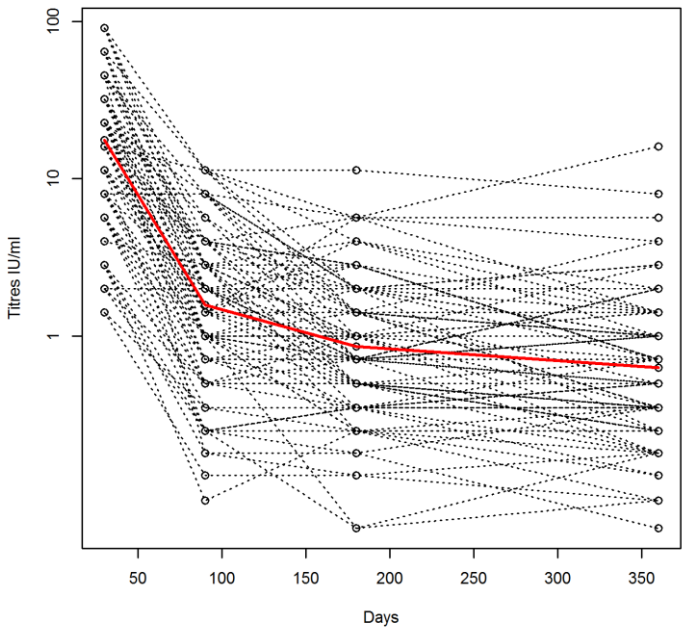

Figure 4.1 Titres of all the dogs (n=82) in the Zenzele research cohort that were blood sampled all at four time points (30, 90, 180 and 360 days after vaccination). Upper outliers (i.e. the seven dogs with day 30 titres ≥128 IU/ml) are excluded. The geometric mean titre is shown in red.

The Bali data contained only one or two data points for each individual (i.e. day 180 and 360), and so instead a linear relationship to time was used (instead of quadratic). To facilitate comparisons with the Bali cohorts, linear models were fitted to truncated data sets for Zenzele (i.e. day 90 or 180 to 360), and the time (points) were adjusted to allow the intercepts to correspond to titres on day 90 or 180 respectively. Experimental studies report a spike in titre immediately following vaccination, followed by a prolonged, slow decline in titre (Bahloul *et al.* 2006). Although a quadratic relationship with time fits the Zenzele data set well over the observed range of the data (Appendix 4.17), it does not monotonically decrease over time and hence is a poor choice for predictions beyond the range of the data. Exponential decay models do decease monotonically, but do not have heavy enough tails given how we would expect the titres to decay outside the range of the data, based on previous studies (Bahloul *et al.* 2006). Fitting alternative models to a skewed distribution with heavy tail for predictions is challenging given that there is insufficient data in the extremes in order to robustly estimate the tail. Therefore, linear models, fitted to Zenzele data sets that exclude peak (day 30) titres, were selected to approximate prolonged, slow declines in titre for predictions in GMT beyond the last time point (Appendix 4.16).

To explore the relationship between the natural log of the day 30 (peak) titres and the covariates described in Section 4.2.3. and lymphocyte and eosinophil counts on day 0, 180 and 360 for Zenzele, linear models were fitted to these data and model selection performed using stepAIC with the MASS package in R (3.0.1) (Venables & Ripley 2002). These models were equivalent to an analysis of variance. An association between eosinophil counts and antibody titres at each time point was also assessed.

The final models were checked for violation of constant variance and normal error distribution assumptions.

Mann-Whitney tests were used to compare titres between (a) vaccinated dogs in Zenzele, Kelusa and Antiga for the same time points, (b) unvaccinated dogs in Kelusa and Antiga for the same time points, and (c) dogs present in Zenzele in May 2006 and those that arrived into the population after May 2006. The natural log of the titre was used for these comparisons. The Mann-Whitney test was also used to compare peak (day 30) titres between dogs with day 360 titres <0.5 IU/ml and ≥ 0.5 IU/ml in Zenzele, and the cube root transformation of the titre was used to stabilise the variance according to a Box-Cox transformation. Population structures were stable (Chapter 2), therefore life expectancies were estimated, by standard

analysis of vertical life tables (Caughley 1977; Pianka 1999; Gsell *et al.* 2012), from the observed ages of the entire study population at the end of the study period.

4.3 Results

4.3.1 General description of the study populations

Almost all of the dogs in the study populations were owned but free-roaming, with <10% confined continuously or frequently during the study period March 2008 – April 2011. There was an approximately even ratio of male to female dogs in Zenzele, but the ratio was skewed towards males (approximately 75%) in Bali. Less than 2% of dogs were sterilized in Zenzele, but castration of juvenile male dogs by community members was common in Bali (approximately 14% in Kelusa and 27% in Antiga) (Chapter 2). Life expectancy was at least 3 years for the majority of dogs in the study populations (Appendices 2.5 and 4.5).

4.3.2 Vaccination coverage

High vaccination coverage was achieved through door-to-door vaccine delivery: 82% (259/315) in Zenzele, 81% (284/351) in Kelusa and 79% (259/327) in Antiga. Similar coverage (75-86%) was achieved in Bali for blood sampling at day 180 and 360, despite many of the dogs having been caught on at least one previous occasion (Appendix 4.2). The characteristics of dogs that avoided capture are described in Appendix 4.6. The sex ratio and age distribution of these dogs were similar to the overall population (Appendix 2.5).

Attrition of the cohorts occurred during the study period through mortality, particularly of neonates, but also through the relocation and disappearance of dogs (Chapter 2). Of the 259 dogs vaccinated in Zenzele at the start of the study, 103 (40%) were sampled at the last time point. Similar proportions were recorded in Kelusa (44%, n=124) and Antiga (49%, n=126) (Appendices 4.2 and 4.3).

4.3.3 Assessment of prior vaccinations

In the Zenzele research cohort, upper outliers were defined as dogs with peak titres (on day 30) of 128 IU/ml or greater (n=7). Some of these dogs were either in the study area in May 2006 or may have been previously independently vaccinated by their owner. Baseline titres of the upper outliers were ≤0.25 IU/ml, most with a titre of ≤0.09 IU/ml. The history of those individuals with the next highest titre (91 IU/ml) varied, and included seven dogs that were born in Zenzele after October 2009.

It is unlikely that any of the dogs vaccinated by the DoA four months prior to initiation of vaccination of the research cohort were inadvertently included in the research cohort. The day 0 titres of the research cohort (including upper outliers ranged from 0.06 - 1 IU/ml with a GMT of 0.1 IU/ml) were substantially lower than the day 90 titres of the DoA cohort (including upper outliers ranged from 0.06 -128 IU/ml with a GMT of 2.8 IU/ml). Thirteen (20%) of the dogs vaccinated by the DoA had titres ≤1 IU/ml 90 days after vaccination, of which 6 had titres <0.5 IU/ml and four of these were non-responders (i.e. day 30 titre of <0.5 IU/ml). Only five dogs in the research cohort had day 0 titres ≥0.5 IU/ml, and of these none appeared to have an anamnestic response to the vaccine (day 30 titres ranged from 1.4 - 45 IU/ml) (Appendices 4.7 and 4.8). There were no differences in the distributions of titres for dogs in Zenzele probably present in May 2006, when the DoA vaccinated, and those that arrived into the population after May 2006 (Appendix 4.9).

In the Bali research cohorts, upper outliers were defined as dogs with day 180 titres of 11.3 IU/ml or greater (n=4 in Kelusa; n=15 in Antiga). For some of these dogs, information provided by owners, breed, source and geographical location was suggestive of vaccination undertaken independently by their owner or as part of vaccination campaigns outside of Kelusa and Antiga. Several (n=15) unvaccinated controls had titres ≥0.5 IU/ml (Appendix 4.10). The titres of the unvaccinated controls are summarised in Appendix 4.11.

4.3.4 Evaluation of antibody titres

4.3.4.1 Titre variations in the vaccinated dogs

The quality of the serum samples was excellent, with only a few samples with slight to moderate haemolysis. Most dogs in Zenzele seroconverted (97% of the research and 92% of the DoA cohorts had titres ≥0.5 IU/ml at day 30), however there was considerable variability in titres at each time point (Figure 4.2). The estimated dog-dog variation (random effect) in peak titres (quadratic model intercept) was large (+/- 2SD 1.8 – 99 IU/ml) (Appendix 4.16, model 1). Excluding upper outliers, the observed geometric mean titres (GMT) at day 30 for the research cohort (of 15 IU/ml, Appendix 4.17) was comparable to experimental (Bahloul *et al.* 2006; Hu *et al.* 2008) and field (Tepsumethanon *et al.* 1991) studies of previously unvaccinated dogs. The maximum peak titre was more than double the upper limit of the other studies (40-50 IU/ml), however those dogs with peak titres >40 IU/ml included seven dogs born in Zenzele after October 2009 which were unlikely to have been vaccinated prior to commencement of the study. There was similar variability in the titres at each time point

for the Bali cohorts (Figures 4.3; Appendix 4.16, models 3-5). See Appendix 4.12 for details of the dogs in Zenzele that did not seroconvert to the vaccine.

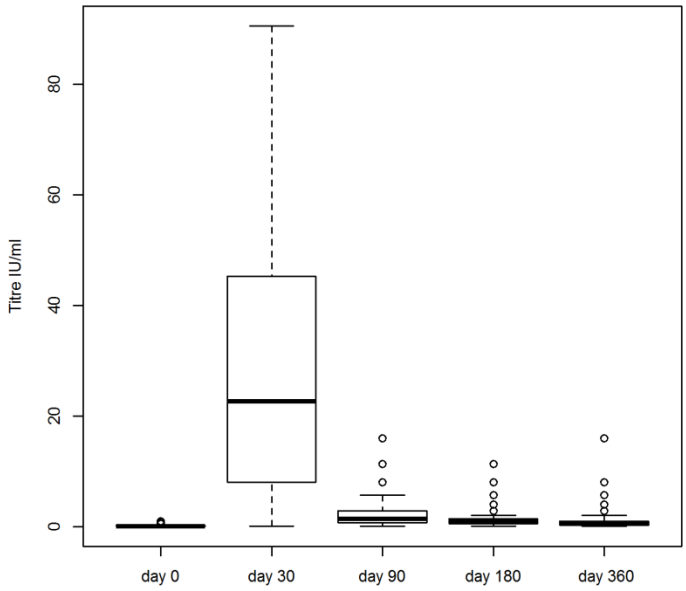

Figure 4.2 Titres of all the dogs in the Zenzele research cohort. Upper outliers (i.e. the seven dogs with day 30 titres ≥128 IU/ml) are excluded. The median titre (thick, horizontal line), 25th and 75th percentiles (thin horizontal lines), and either minimum and maximum titres or 1.5x the interquartile range (dashed vertical lines) are shown for each time point after vaccination (at day 30, 90, 180 and 360). Day 0 shows the distribution of titres immediately prior to vaccination.

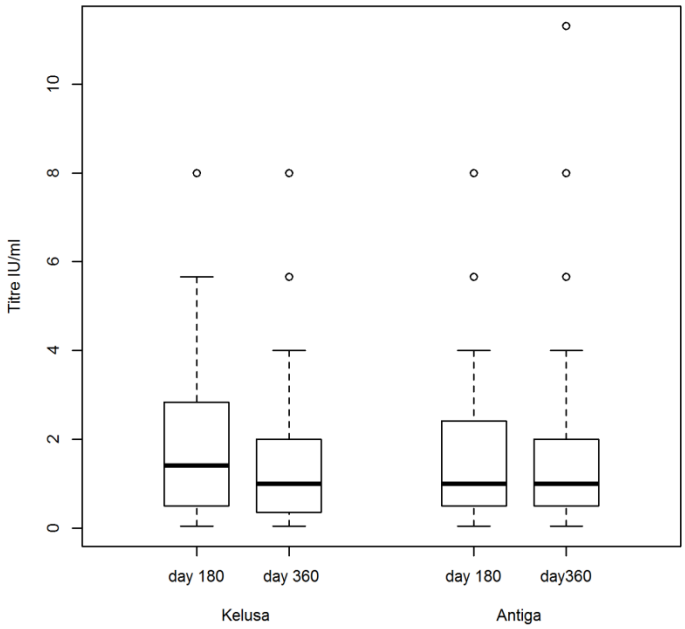

Figure 4.3 Titres of all the dogs in the Kelusa and Antiga research cohorts. Upper outliers (i.e. the four dogs in Kelusa and fifteen dogs in Antiga with day 180 titres ≥11.3 IU/ml) are excluded. The median titre (thick, horizontal line), 25th and 75th percentiles (thin horizontal lines), and either minimum and maximum titres or 1.5x the interquartile range (dashed vertical lines) are shown for each time point after vaccination (at day 180 and 360).

Although the GMTs for the Bali cohorts were statistically significantly higher than Zenzele (Mann-Whitney test $p \leq 0.05$) for day 180 and 360, the value of the means and modes were comparable between cohorts at each time point (Appendix 4.13). Less than 10% of each cohort had titres of ≤0.1 IU/ml at day 360. For Zenzele, peak titres did not exceed 5.7 IU/ml for these dogs, and three were non-responders. See Appendix 4.14 for details of the dogs in each cohort with titres ≤0.1 IU/ml 360 days after vaccination. Between 20-40% of dogs overall had titres <0.5 IU/ml at the last time point. Excluding upper outliers, dogs in Zenzele with day 360 titres <0.5 IU/ml had a statistically significantly (Mann-Whitney test $p<0.001$) lower day 30 GMT (6.6 IU/ml, n=38) compared to dogs with day 360 titres ≥0.5 IU/ml (23.6 IU/ml, n=57); this is consistent with the correlations between time points discussed below.

4.3.4.2 Kinetics of titres in the vaccinated dogs

In Zenzele, titres declined rapidly between day 30 and 90, then gradually from day 90 (Figures 4.1 and 4.2). Log titres were closely correlated across all the time points, including between day 30 and 360 (excluding outliers correlation coefficient $r = 0.55$) and day 30 and the mean log titre for day 90, 180 and 360 ($r = 0.72$) (Appendix 4.15). Consequently, dogs

with higher peak titres tended to have higher titres at the final time point. The model assuming a quadratic relationship between titre and time was a good fit, with predicted GMT for the day 30 (peak) and 360 titres congruent with the observed means (Appendix 4.16, model 1; Appendix 4.17).

Peak (day 30) titres could not be extrapolated from the linear models for Bali, but the decline in titres between 180 and 360 in Kelusa was similar to Zenzele (Appendix 4.16, models 2-4). The GMT for Antiga declined only marginally with time (slope p=0.4; Appendix 4.16, model 5), with a rate of decline less than quarter that of Zenzele and Kelusa. Overall, the predicted GMTs were comparable to the observed titres (Appendix 4.16, models 1-5; Appendix 4.17).

The magnitude of any decline in titre as a consequence of extended storage time or freeze-thaw cycles was not great compared to normal background variation. This agrees with other studies evaluating the effect of storage time and freeze-thaw cycles on blood proteins (Thoresen *et al.* 1995; Reynolds *et al.* 2006). The decline in titre for 22 (88%) of the samples did not exceed normal inter-assay variation of two-fold or less (Gilbert *et al.* 2013).

4.3.4.3 Factors associated with variations in titre

When comparing the research cohorts, all models with time (points) had lower AICs than the null models except for Antiga when upper outliers were excluded, indicating that time after vaccination had an effect on titre. While there were no clear patterns across the cohorts between variations in titre and the covariates described in Methods and materials section 4.2.3., lactation and health status emerged as significant covariates.

For Zenzele, apart from lactation at vaccination, there were no statistically significant ($p<0.05$) associations of titre with age, gender, reproductive and health status, and body condition when accounting for all the time points (i.e. for models of the longitudinal data). Time and lactation were the only covariates retained in the quadratic model with the lowest AIC, where the negative effect of lactation was statistically significant ($p\leq0.02$) (Appendix 4.19, model 1). When the response variables was restricted to peak (day 30) titres, titres again varied significantly with lactation ($p\leq0.02$) (Appendix 4.19, model 2). Overall, the GMT of lactating dogs (~6 IU/ml) was less than half that of males and non-lactating females. Clinical signs at the time of vaccination was also significant (p=0.04) when the response variable was restricted to peak (day 30) titres but only when upper outliers were included in the model (Appendix 4.19, model 3). Those dogs with clinical signs at the time of vaccination had a GMT of 11 IU/ml, approximately half that of dogs without clinical signs (21 IU/ml). When

body condition was dropped from the model, clinical signs at the time of vaccination was marginally statistically significant (p=0.06) (Appendix 4.19, Note 4); the differences in factor levels (i.e. with and without clinical signs at vaccination) between the two models were comparable (9.4 IU/ml including and 8.0 IU/ml excluding body condition).

Lactation and health status at vaccination were also the main covariates of significance in the Bali villages. Lactation and pregnancy were excluded from the models for Kelusa (Appendix 4.20) given their small group sizes (Appendix 4.23) and these covariates were not retained in any of the models for Antiga (Appendix 4.21). However, a positive effect of lactation at vaccination was marginally statistically significant (p=0.07) to statistically significant (p=0.05) for Kelusa and Antiga combined when upper outliers were included in the model (Appendix 4.22, model 1 and Note 2).

When generalised dermatitis at the time of vaccination was included in the combined model, lactation was no longer significant (Appendix 4.22, model 2). Generalised dermatitis was statistically significant ($p \leq 0.02$), although the GMT was only approximately 0.5 IU/ml less than the baseline (i.e. dogs not lactating without dermatitis GMT ~2 IU/ml). Generalised dermatitis was retained in models for Kelusa, but it was not statistically significant (p=0.15) (Appendix 4.20, model 1) even though 44 (37%) of dogs at the time of vaccination were affected (Appendix 4.23). However, generalised dermatitis at the time of vaccination was generally highly statistically significant for the Antiga cohort ($p \leq 0.01$) reducing the GMT by up to half (Appendix 4.21, models 2 and 3).

Convergence errors, regardless of fitting method, precluded full evaluation of the data set combining all three research cohorts.

4.3.4.4 Rabivet Supra 92

Of the 16 dogs vaccinated in December 2009 with Rabivet Supra 92 all had titres <0.5 IU/ml except for one dog at day 180 and two at day 360 sampling (Appendix 4.24).

4.3.4.5 Lagos Bat Virus assays

The samples were negative for the Zenzele cohort. All the samples were negative for the Bali cohorts except for one dog in Kelusa, with a 50% end-point titre of 1/64 upon initial testing and 1/32 upon re-testing.

4.3.4.6 Other diagnostics

Lymphocyte counts were significantly associated with body condition at the time of vaccination (p=0.03) (Appendix 4.19, model 4), however there was no association with peak (day 30) titres (p>0.05). There were no associations between eosinophil counts and titres at any time point.

Almost all of the dogs had intestinal parasites, primarily *Ancylostoma spp.* (Appendix 4.25) (Minnaar, Krecke & Rajput 1999; Minnaar & Krecke 2001). Consequently, there was insufficient variability to determine the effect of intestinal parasites on immune response to vaccination. One dog was positive for *Demodex spp.* on deep skin scrape.

4.4 Discussion

The longitudinal, individual-level data from this study provides the most detailed serological data currently available for domestic dogs in rabies endemic areas, and provides valuable support for planning rabies vaccination programmes.

This study reinforces the importance of frequent and regular vaccination campaigns to ensure effective vaccination coverage is maintained. Dogs with lower peak titres had correspondingly lower titres at the end of the study, with titres <0.5 IU/ml at the last time point (day 360) for 20-40% of the dogs and <0.1 IU/ml for 3-8% of the dogs (Appendix 4.13); the implication being an increased susceptibility to natural exposure with time in the dogs with low titres (Precausta *et al.* 1985; Aubert 1992; Bahloul *et al.* 2006; CDC 2008; Siegrist 2008). Robust demographic data from these study populations indicates, two years after a pulse campaign which achieved 80% vaccination coverage, at least 20-45% vaccination coverage would remain (Chapter 2), which is the critical threshold necessary to prevent rabies (Hampson *et al.* 2009). However, from our model predictions (Appendix 4.16), we speculate that a substantial proportion of the dogs remaining in Zenzele two years after vaccination may have titres <0.1 IU/ml, potentially dropping effective vaccination coverage to below the critical threshold. Models were constrained to two time points for the Bali cohorts, but predicted similar declines in the GMT for Kelusa.

The vast majority of the dogs seroconverted following vaccination (with a peak titre of ≥0.5 IU/ml), regardless of health status. However, there was considerable variation in titres at each time point for all the cohorts. Peak titres were not measured for the Bali cohorts, however day 180 titres were comparable to Zenzele, therefore it is likely that a similar proportion of dogs

to Zenzele seroconverted following vaccination. Identification of risk factors associated with lower titres may promote targeted boostering to maintain vaccination coverage. Clinical conditions around the time of vaccination reduced the immune response to the vaccine in all the cohorts; in particular, generalised dermatitis provided a 'visible marker' for a reduced immune response, with practical implications for rabies control. While demodicosis was assumed to be an important cause of generalised dermatitis associated with immuno-suppression in Bali, the mostly negative skin scrapes suggests that dermatophytosis may be more likely, consistent with both the tropical climate and immuno-suppression (Guaguere, Prelaud & Craig 2008; Miller, Griffin & Campbell 2013). This warrants further investigation given that a substantial proportion of the dogs (37%-46%; Appendix 4.23) were affected, potentially reducing the effectiveness of vaccination. Lactation at the time of vaccination in Zenzele and the Bali cohorts combined was marginally significant statistically, however its biological significance is unclear. Lactation is associated with loss of body condition in all the research sites (Chapter 2), consistent with immuno-suppression observed in Zenzele. The reason for the opposite effect in Bali cannot be readily explained (Lloyd 1983; Lloyd, Amerasinghe & Soulsby 1983). While this incongruity may warrant further investigation in larger study populations, on balance lactating bitches should be vaccinated, with re-vaccination following weaning.

Our study demonstrated an advantage of community engagement and door-to-door vaccine programmes over the use of simple vaccination points. We consistently achieved vaccination coverage above 70% through door-to-door vaccine delivery, even in Bali where the majority of the dogs needed to be caught by net on successive occasions. Similar coverage was achieved across the rest of the island through door-to-door vaccine delivery in 2010 and 2011 (Putra *et al.* 2013). This compares to a vaccination coverage of only 27% through the vaccination point in Zenzele and a very low vaccine uptake (5%) in Kelusa. The utility of vaccination points is likely to differ between locations according to local circumstances. Similar to other communities in Africa, Europe and central Asia where free-roaming dogs are handleable (Bogel & Joshi 1990; Cleaveland *et al.* 2003; Kaare *et al.* 2009; Lembo *et al.* 2010), it is likely that the majority of the dogs in Zenzele could have been delivered to the vaccination point by their owners, and the low vaccination coverage was probably the result of inadequate advertising (Durr *et al.* 2009) and limited operating hours during a work / school day. Vaccine uptake in Kelusa was, in part, affected by community awareness of the research vaccination programme, however the majority of the dogs could not be handled by their owners or the vaccinators, thus necessitating restraint by net (Putra *et al.* 2013). The

reasons for the difference in handleability between locations are unclear. Restraint by net is more stressful to the dog, time consuming and costly than by leash and muzzle. In order to improve welfare, facilitate more cost-effective and efficient delivery of vaccines (and other prophylactics), and improve evaluation of the dogs in Bali and similar communities, extending our studies to evaluate the differences in husbandry, environment and other factors influencing the temperament of the dogs in the sites is warranted.

This research has generated valuable data that may contribute to rabies control, including through improving epidemiological models. However, understanding variation between dogs in titres measured from field studies is challenging. Some covariates which may impact on titres, such as lactation and health status, are measurable, whereas others such as genetics and stress are harder to assess in real time. Further evaluation of factors associated with variation in immunity over time since vaccination, including both serological responses and direct assessment of CMI, and recording vaccine failures is warranted and may require larger populations studied and over longer time periods.

4.5 Conclusion

This study demonstrates that the vast majority of free-roaming dogs, in two regions of Africa and Asia where rabies is endemic, seroconverted to rabies vaccine regardless of health status producing titres that exceeded 0.5 IU/ml, the level considered necessary to protect against rabies. Declines in vaccination coverage following a vaccination campaign occur through mortality / emigration of vaccinated dogs and birth / immigration of unvaccinated, susceptible dogs. Robust demographic data from the study populations show that two years after vaccinating at least 70% of dogs during a pulse vaccination campaign, vaccination coverage remained within 20-45% (Chapter 2), the range necessary to control rabies (Hampson 2009). However, our serological data indicates that dogs with lower peak (day 30) titres had correspondingly lower end point (day 360) titres. We speculate that a proportion of vaccinated dogs remaining in the study populations after two years will probably have titres below the approximate threshold for protection (<0.1 IU/ml) thus dropping effective vaccination coverage to below the critical threshold (of 20-45%). This emphasizes the importance of re-vaccinating within two years. Vaccination of all dogs during annual campaigns is therefore recommended as the most effective means of ensuring that individual immunity and population coverage are both maintained at sufficient levels to control rabies.

Chapter 5

Modelling human interference and rabies transmission in free-roaming dogs

Summary

Deterministic, compartmental models have been used to explore local transmission dynamics of canine rabies, particularly for the development and implementation of control measures. These models have assumed density-dependent transmission and included demographic parameters limited to births and deaths derived from samples of owned dogs at the district or city level. More recent demographic and epidemiological evidence from the field justifies re-evaluation of local transmission processes of canine rabies, including the possible effects of human interference (i.e. through the killing or translocation of diseased dogs) on transmission.

We apply a stochastic compartmental model to explore the transmission dynamics of rabies in a range of realistic, free-roaming dog populations. We model the effect of human interference through assuming that the removal rate of infected dogs scales with human, and thus dog, population density. We also investigated the value of incorporating individual-level demographic data into the model. We show that empirical attack rates suggest that the basic reproductive number (R_0) in local populations (e.g. at the village level) may be <1 and lower than the previously estimated global R_0 (e.g. at the provincial level). We find that even a low rate of humans bringing infected dogs into a local population may off-set the benefits of high vaccination coverage. These findings suggest that human factors should be considered in epidemiological modelling and disease control for canine rabies; and, regular, thorough vaccination campaigns at the local level are essential, especially in the presence of interventions that may increase human-mediated movement of dogs, such as mass sterilization programmes. Finally, we found no clear advantage of individual-based models over simpler compartmental models with exponential age distributions.

5.1 Introduction

Canine-mediated rabies is a serious zoonosis, responsible for at least 55,000 human deaths per year (Knobel *et al.* 2005). Understanding the transmission dynamics of the disease is essential for its control. Epidemiological models, based on observational data, have been used to explore spatial dynamics (Beyer *et al.* 2010; Beyer *et al.* 2012; Townsend *et al.* 2013b) and local transmission processes (Cleaveland & Dye 1995; Kitala *et al.* 2002; Hampson *et al.*

2007; Zinsstag *et al.* 2009), particularly to inform the development and implementation of control measures. The local transmission processes have been evaluated using deterministic, compartmental models, including demographic parameters (births and deaths only) derived from samples of households at the district or city level, and transmission parameters from observations of natural infections. These models assume density-dependent transmission, whereby the population is "well-mixed" and encounters between susceptible and infectious individuals increase with population density (Keeling & Rohani 2008). Under this assumption, disease incidence is expected to increase with host density, as will the basic reproductive number R_0, an important epidemiological parameter that indicates whether an outbreak will spread. R_0 is defined as the average number of secondary infections produced when one infected individual is introduced into a wholly susceptible population (Anderson & May 1991). For a disease to spread epidemically, R_0 must exceed the threshold value of 1. For simple, "well-mixed" models R_0 depends on the rate of transmission and the infectious period, and the expected outbreak size depends on R_0. However, as discussed below, more recent empirical evidence (Hampson *et al.* 2009) (Chapter 2) justifies re-evaluation of local transmission processes, including the possible effects of human interference on transmission, which relates to complex interactions between people and dogs (Chapter 1). Currently these effects are poorly understood; however exploratory models based on realistic free-roaming dog populations, that account for human interference, may provide new insights into local disease dynamics and, thus, direct future field research and enhance rabies control policy.

Canine rabies can be controlled effectively by vaccination (Cleaveland *et al.* 2003; Belotto *et al.* 2005; Schneider *et al.* 2005; Cleaveland *et al.* 2006; WHO 2013). Vaccination campaigns are often undertaken annually and aim to achieve 70% coverage of the dog population (WHO 2013). Empirical evidence and theory suggests that a vaccination coverage of 70% should maintain population immunity above the critical levels (20-45%) required to interrupt rabies transmission (Coleman & Dye 1996; Hampson *et al.* 2009). A vaccination coverage of 20-45% is consistent with a low R_0 (<2), estimated from empirical data of early exponential growth of epidemics in dog populations (Heffernan, Smith & Wahl 2005; Wallinga & Lipsitch 2007) across a wide geographic range (Hampson *et al.* 2009; Townsend *et al.* 2013b). In these studies, R_0 was generally estimated at the global level (i.e. over large geographic areas such as cities or districts) and was consistently <2 (ranging from 1.05-1.72) irrespective of location, suggesting that transmission may be independent of population density. An R_0 <2 is also consistent with a low incidence, or average monthly attack rate, of canine rabies compared to other species (Bogel *et al.* 1974). Attack rates of canine rabies

have also generally been estimated at the global level and are typically <0.5% and rarely exceeds 2% (Waltner-Toews *et al.* 1990; Windiyaningsih *et al.* 2004; Rothman, Greenland & Lash 2008; Zinsstag *et al.* 2009; Tenzin *et al.* 2010; Putra *et al.* 2011; Tenzin *et al.* 2011; Townsend *et al.* 2013a; Townsend *et al.* 2013b). Incidence data in local populations (e.g. at the village level) is limited, although the outbreak size distribution has been reported for the village level in the Serengeti, Tanzania, with a median outbreak size <5 cases and upper limit of approximately 35 cases (Hampson *et al.* 2009). While an R_0 of 1.2 was estimated from outbreak data at the district level for the Serengeti, R_0 at the village level was not reported.

The apparent lack of relationship between R_0 and population density is most consistent with frequency-dependent transmission, where the rate of transmission is assumed to be independent of host density. In this case, "well-mixed" models with frequency-dependent transmission predict host extinction for fatal diseases, such as rabies, as soon as R_0 exceeds unity (Keeling & Rohani 2008). However, this prediction is inconsistent with the very low attack rates reported for dogs. Alternatively, a consistently low R_0 (<2) irrespective of dog population density may be explained, in part, by selective removal, or the swift identification and killing of infectious and in-contact dogs by local communities (Beran 1982; Ogunkoya, Will & Ezeokoli 1984; Ezeokoli & Umoh 1987; Pastoret & Brochier 1998; Belotto *et al.* 2005; Hampson *et al.* 2009; Tenzin *et al.* 2011; Townsend *et al.* 2013b). Selective removal reduces the effective infectious period in dogs (Hampson *et al.* 2009) which may limit outbreak size (Pastoret & Brochier 1998; Tenzin *et al.* 2011), reducing disease incidence, R_0 , and the vaccination threshold, and exacerbate stochastic fade-out, particularly in small populations (Lloyd-Smith *et al.* 2005). We have hypothesized that selective removal may scale with density concealing the existence of density-dependent transmission (Chapter 1). The basis of this hypothesis is that rabid dogs may be spotted and selectively removed from areas with more people present and that human and dog densities are expected to correlate (Oboegbulem & Nwakonobi 1989; Matter *et al.* 1998; Butler & Bingham 2000). Therefore, if the effective infectious period, as reduced by selective removal, scales inversely with human, and thus dog, population density R_0 will be relatively invariant irrespective of population density. This is consistent with simple epidemic model predictions of exponential growth of diseased individuals (I) early in an epidemic:

$$I(t) = I(0)\exp((R_0 - 1)(\gamma + \mu)t).$$

The exponential growth rate is therefore

$$(R_0 - 1)(\gamma + \mu)$$

and not only depends on the value of R_0 but the removal rate (γ) and turnover rate (μ) for the population (Keeling & Rohani 2008); thus, the number of diseased individuals in a population may scale with R_0 or the removal rate. Given the variable but often protracted latent period of rabies (Jackson 2013), demographic processes may also contribute to disease control through the loss of individuals incubating the disease from local populations.

The positive effect of human interference, and stochastic and demographic processes, on the control of transmission in local populations, may be off-set by the influx of infected dogs mediated by people. Human-mediated movement of dogs (some of them infected) may be substantial (Hill 1985; Chomel *et al.* 1987; Beran & Frith 1988; Matter & Fico 1998; Estrada *et al.* 2001; Kongkaew *et al.* 2004) and contribute to the spatial spread of rabies and local persistence through reintroductions (Wells 1954; Shone 1962; Beran 1982; Robinson *et al.* 1996; Denduangboripant *et al.* 2005; Coetzee & Nel 2007; Kasempimolporn, Jitapunkul & Sitprija 2008; Zinsstag *et al.* 2009; Beyer *et al.* 2010; Talbi *et al.* 2010; Townsend *et al.* 2013b). This may be exacerbated by negative community reactions to culling (i.e. the widespread killing of dogs regardless of infection status) (Beran & Frith 1988; Windiyaningsih *et al.* 2004) and sterilization (WHO 2004; Reece & Chawla 2006; Totton 2009), as discussed below. Both culling and sterilization aim to reduce host density in order to reduce disease incidence, even though there is no evidence that the incidence of rabies increases with host density in dog populations (Coleman & Dye 1996; Kitala *et al.* 2002; Hampson *et al.* 2009; Townsend *et al.* 2013b). Sterilizations have been assumed to extend vaccination coverage by reducing the number of new, susceptible dogs entering the population through reducing local births and increasing the longevity of sterilized dogs; although the empirical evidence for this is equivocal (Totton 2009; Totton *et al.* 2010; Jackson 2013). Sterilizations have also been advocated to reduce the proportion of dogs under 12 months of age given that a proportionally higher incidence of rabies is often reported in this age class (Belcher, Wurapa & Atuora 1976; Malaga, Lopez Nieto & Gambirazio 1979; Beran 1991; Mitmoonpitak, Tepsumethanon & Wilde 1998; Widdowson *et al.* 2002); even though it may be simpler to increase vaccination coverage in young dogs (Appendix 6.1), which may be comparatively lower (Cleaveland *et al.* 2003; Flores-Ibarra & Estrella-Valenzuela 2004; Awoyomi, Adeyemi & Awoyomi 2007). However, given increasing evidence that the vast majority of free-roaming dogs in most societies globally are owned

(WHO & WSPA 1990; Cleaveland & Dye 1995; Robinson *et al.* 1996; Butler & Bingham 2000; Estrada *et al.* 2001; Windiyaningsih *et al.* 2004; Gsell *et al.* 2012) and that population size is regulated by the demand for dogs (Chapter 2), killing owned dogs and reducing the local supply of puppies available to meet the demand for dogs may perversely precipitate an influx of dogs into local populations. For example, within a few days of a village-wide cull in Kelusa, Bali Province, Indonesia, two residents brought in unvaccinated, potentially infected puppies from outside the village to replace their culled, vaccinated adult dogs. Community responses to culling and sterilization, in particular their effect on the movement of dogs, is currently being monitored in Kelusa and Antiga, Bali.

This study aims to explore the local dynamics of canine rabies in realistic free-roaming dog populations using simple epidemic models that account for human interference (both the selective removal and movement of dogs) in transmission. We use robust demographic data (Chapter 2) to build an individual-based model for rabies transmission in dog populations. We model the effect of human interference through assuming that the removal rate of diseased dogs scales with human, and thus dog, population density; and investigate whether outbreak dynamics in local populations are consistent with reported estimates of R_0. We also evaluate the effect of a variable influx of infected dogs by people, under different vaccination coverages, on outbreak size and frequency with a view to the evaluation of culling and sterilization in Bali villages on the implications of dog movement on rabies control. Comparisons are made with a simpler stochastic, compartmental SEI (Susceptible-Exposed-Infectious) model to assess the value of incorporating individual-level demographic data into the model.

5.2 Methods and materials

5.2.1 Demographic data

Detailed demographic data were collected from four research sites, two in Gauteng Province, South Africa and two in Bali Province, Indonesia from March 2008 until April 2011. The research sites were part of a larger ecological study (Chapter 2), and were selected based on specific criteria, which included the absence of previous dog population management interventions by local animal welfare non-governmental organizations (NGOs) or authorities. Rabies outbreaks occurred in Bali Province in 2008 and Gauteng Province in 2010.

The two sites in Gauteng Province, South Africa comprised the informal settlement Zenzele west of Johannesburg (26.15^{o}S and 27.41^{o}E) and Braamfischerville in Soweto (26.12^{o}S and

27.52°E). The study area encompassed the entire Zenzele township, whereas approximately one third of Braamfischerville was included in the study area to include a comparable number of dogs to Zenzele. In Indonesia, the two sites included the villages of Kelusa (8.26°S and 115.15°E) and Antiga (8.30°S and 115.29°E) on the island of Bali. In Kelusa, the study area encompassed the entire village with the exception of Banjar (sub-village) Yehtengeh, which is separated from the rest of the village by rice fields and jungle, the southern half of Banjar Kelikikawan and households scattered along the main road leading into the village. In Antiga, the study area encompassed all of the main residential area (Banjars Kaler and Kelod). An additional area within Banjar Ketug included entrances scattered along a 2.7 km stretch of road winding through the jungle north of Kaler and Kelod. All households in the study areas were included in the sampling frame.

Individual-level data for every identified dog in the study area were collected longitudinally by direct observation and questionnaire through door-to-door surveys every 6-12 weeks during the study period (of 36 months in Zenzele and Braamfischerville, 34 months in Kelusa and 38 months in Antiga). The study population comprised of every owned dog (i.e. dog belonging to a household in the study area). Pups were defined being in their 1st – 3rd month of life (i.e. 0 – ~13 weeks of age), juveniles 4th – 12th month of life (i.e. ~14 – 52 weeks of age), and adults older than their 12th month of life. The demographic data included in the models comprised of all owned dogs in the third month of their life or older in the study areas. The majority (>90%) of dogs were free to roam intermittently or continuously in Zenzele, Kelusa and Antiga, whereas in Braamfischerville approximately 40% of dogs were confined most of the time. Therefore, in addition to owned dogs being monitored at their household, every dog encountered in a yard not their own or on the street during each census was identified as either belonging to a household in the study area or not. Each dog identified as not belonging to a household in the study area was classified as unowned. Only two dogs in Johannesburg and eighteen dogs in Bali were identified as unowned; these dogs were not included in the study population.

The date each dog was acquired was reported by owners, and for most dogs the date was re-recorded at least once during the study period. The date each dog was lost from the study area was generally only recorded once. The month of loss was reported by the owner for 68% of the dogs in Zenzele, 78% in Braamfischerville, 51% in Kelusa and 55% in Antiga. The remainder were randomly allocated to a month between the census or revisit in which they were last recorded and the subsequent one. The origin and outcome of each dog was also recorded.

Human demographic data were collected in Zenzele and Braamfischerville during eight and six of the door-to-door surveys respectively, and in Kelusa and Antiga during seven of the door-to-door surveys (including the first and last surveys for all of the sites). The same data were also collected during one survey in 2009 from a random sample of 80 and 141 non-dog owning households in the Zenzele and Braamfischerville respectively; and from all non- and ex-dog owning households during one survey in 2009 in the Bali sites (a total of 50 households in Kelusa and 99 households in Antiga).

5.2.2 Model structure and parameters

Rabies is a fatal disease, generally with a long latent period (Jackson 2013) between infection and infectiousness. We therefore consider models with susceptible (S), exposed (E) and infectious (I) states. The models depend on three key epidemiological parameters for rabies: the transmission rate (β), the latent period ($1/\sigma$) and the infectious period ($1/\gamma$) (Tables 5.1 and 5.2).

Table 5.1 Transitions and their probabilities in the individual-based and compartmental models.

description	transition	probability of transition in time δt
a dog becomes exposed to the virus	S → E	$(\beta \mathrm{SI})\delta t$
a dog becomes infectious	E → I	$\sigma \mathrm{E} \delta t$
a dog dies from the virus	I → ∅	$\gamma \mathrm{I} \delta t$

N is the total number of dogs in the population at any one time (i.e. N = S+E+I)

Table 5.2 Demographic events and their probabilities in the compartmental model.

description	event	probability of an event in time δt
a susceptible dog is gained into the population	$\emptyset \rightarrow S$	bN
a susceptible dog is lost from the population	$S \rightarrow \emptyset$	μS
an exposed dog is lost from the population	$E \rightarrow \emptyset$	μE
an infectious dog is lost from the population	$I \rightarrow \emptyset$	μI
a vaccinated dog is lost from the population	$V \rightarrow \emptyset$	μV

b = the mean number of dogs gained per capita per day, which is assumed to = μ [the mean number of dogs lost per capita per day]

The exact number of dogs gained (from all sources) and lost (for all reasons) for each study population were known (see section 5.2.1.). The study populations are small (average of <350 dogs in each community; Table 5.3) therefore the model is stochastic, which is the preferred method for modelling outbreak dynamics in small populations (Keeling & Rohani 2008). The majority of the dogs in the populations were free-roaming consistent with the model assumption of "well-mixed" populations. For the individual-based model population size was not fixed and the demography was deterministic, updating the population by removing and adding dogs lost and gained from the study population at each (monthly) time step (δt). For the compartmental model, the loss rates were fixed at average values derived from the demographic data. The population size therefore fluctuates around the initial value.

Table 5.3 Demographic parameter estimates for the study populations.

location	area A_L (km^2)	mean population size N	population size at time 0	mean number of dogs lost per capita per day μ
Zenzele	0.72	323	349	0.00208
Braamfischerville	1.26	283	288	0.00195
Kelusa	0.66	287	252	0.00156
Kaler (Antiga)	0.30	175	166	0.00148
Ketug (Antiga)	0.63	82	81	0.00140

Both models assume constant latency and removal rates, with the latent and infectious periods exponentially distributed. Empirical studies of these distributions indicate that they are less dispersed than predicted by exponential distributions and potentially better described by gamma distributions (Hampson *et al.* 2009). However, we have no information or intuition on how the dispersion of infectious period distributions may scale with population density. Therefore, for simplicity we only explore the constant rate (exponential) model here. The compartmental model is simulated by the (Gillespie) Stochastic Simulation Algorithm. The individual-based model uses a modified form of the Gillespie algorithm, where a demographic event is defined before the Markov simulation. At each time step (δt) a Markov simulation is attempted and carried out only if no demographic (non-Markov) events occur during the time step.

When the average latent period is small compared to the average life expectancy, as for canine rabies, then the expression for R_0 for an SEI model reduces to that for an SI model (Keeling & Rohani 2008). Therefore, we used equation 1 to estimate R_0.

$$R_0 = (N\beta_L + i)/(\gamma[n] + \mu) \qquad \text{equation 1}$$

Transmission is density-dependent however, to account for a relatively invariant R_0 irrespective of local population density consistent with empirical data (Hampson *et al.* 2009), the removal rate (γ) was scaled according to the average dog density (i.e. dogs km^{-2}) for each study population (n) as proxy for human density. The overall removal rate (γ) in the model is composed of the removal rate without human interference (m), fixed as the reciprocal of the average infectious period of 3.7 days without selective removal (Hampson *et al.* 2009), and the removal rate that scales with the average study population density (α) (equation 2). This generates a lower bound in population density below which selective removal does not occur.

$$\gamma = m + \alpha[n] \qquad \text{equation 2}$$

The transmission rate (β_0) was initially calculated in units of population per units of area (i.e. dogs per km^2 per day) (see below). In order to parameterize the stochastic model with a transmission rate in per capita units (dog.days) and average study population size (N) (rather than average study population density), β_0 is adjusted by the local study area (A_L) (i.e. km^2) (equation 3). The average loss rate per capita per day and the number of infected dogs entering the population (from outside the population) per day (i) are small and, thus, ignored when calculating removal rates.

$$\beta_L = \beta_0 / A_L \qquad \text{equation 3}$$

A baseline transmission rate (β_0) of 0.0469 dogs per km^2 per day was derived from the most robust empirical data reported, obtained through contact tracing, in the Serengeti and Ngorongoro Districts in Tanzania (Hampson *et al.* 2009). This study estimated a global R_0 of 1.19 (from the initial epidemic growth curve) for the Serengeti and an infectious period of 3.1 days, accounting for selective removal. An average global dog population density (of 8.19 km^{-2}) was estimated for these those two districts combined, based on the distribution of reported outbreaks for the districts. A map of the region indicates heterogeneous dog population density (Lembo *et al.* 2010), and the average dog density at the village level may be substantially higher than the global density. Therefore, transmission rates of 0.0076 dogs per km^2 per day and 0.0038 dogs per km^2 per day were recalculated on the basis that the average local dog density across the region was arbitrarily 50 and 100 dogs km^{-2} respectively. Although the latent period is highly variable (Jackson 2013), the average latent period was fixed at 25 days consistent with the empirical data (Hampson *et al.* 2007; Hampson *et al.* 2009). An R_0 of 1.2 was also estimated for Bali from the initial growth curve of the 2008 epidemic (Townsend *et al.* 2013b).

5.2.3 Model scenarios

An outbreak is defined as at least two cases not interrupted by an interval of more than one month, as per Hampson *et al* (2009). Single cases that arise from seeding with an exposed dog and exposed seeds that do not transition to the infectious state (i.e. infected individuals that are lost from the population through demographic processes) are defined as index cases. The probability of an outbreak and median outbreak size and the quantiles were estimated from distributions of 1000 simulations for each of the following scenarios:

5.2.3.1 Comparison of outbreak dynamics, R_0, and transmission rates

We investigate whether outbreak dynamics in the study populations are consistent with reported estimates of R_0 for canine rabies. We also explore the sensitivity of the model for the range of R_0 and transmission (and removal) rates. We use a range of R_0 (from 0.5 to 1.2) and transmission rates (0.0469, 0.0076 and 0.0038 dogs per km^2 per day area adjusted [β_L]) for each study population, with models seeded with a single exposed dog at the start of the time series. Models are seeded with exposed dogs on the assumption that most local incursions

occur through human-mediated movement of an infected (not infectious) dog, and a proportion of these dogs will be lost from the population before becoming infectious.

5.2.3.2 Probability of an outbreak with vaccination coverage

In order to benchmark how well simple demographic models describe the rate of decline in vaccination coverage we simulate the probability of an outbreak for a range of vaccination coverage (from 0% to 80%) for an R_0 of 0.7 and 1.2 and the maximum and minimum transmission rates (0.0469 and 0.0038 dogs per km^2 per day area adjusted [β_L]) for each population. Vaccination coverage was calculated for the total number of dogs present in the population at the start of the study period for the individual-based model and for the mean population size for the compartmental model (Table 5.3). Models are seeded with a single exposed dog at the time of vaccination (at the start of the time series) and 12 and 24 months following vaccination. Outbreak size was not estimated because truncation of the time series precluded proper comparisons between the time points.

Estimates of vaccination coverage in the study populations were obtained by assigning a random sample of dogs from the starting cohorts equal in size to the proportion assumed to be vaccinated, and determining those still present at 12 and 24 months. This process was repeated 1000 times to produce Monte Carlo estimates of the drop-off in vaccination coverage.

5.2.3.3 Comparison of outbreak dynamics, human-mediated incursions and vaccination coverage

We evaluate the effect of human-mediated incursions of infected dogs on outbreak dynamics in the Bali populations for a range of vaccination coverage (from 0% to 80%) at the start of the time series using the individual-based model. We use parameter values that are biologically plausible for local transmission derived from the initial analysis described under 5.2.3.1 Comparison of outbreak dynamics, R_0 , and transmission rates. These values include an R_0 of 0.7 and area adjusted transmission rates of 0.0076 and 0.0038 dogs per km^2 per day [β_L]. The probability of a human-mediated incursion was estimated from recent outbreak (Townsend *et al.* 2013b) and geographic (Putra *et al.* 2011) data for Bali and demographic data for the study populations (Chapter 2). Townsend et al (2013) estimated the probability of human-mediated transport of dogs across the island to be 0.05-0.09. This estimate agrees with the observed proportion of dogs relocated by people out of Kelusa and Antiga during the study period. The probability of human-mediated transport of dogs of 0.05 was combined

with the outbreak (incidence) and geographical data for Bali and the observed distribution of dogs gained into each study population. This was to estimate the baseline probability that a dog gained into the study population at some point during the study period was exposed. This baseline probability applies to a study population subject to rabies but not population controls, such as sterilizations.

Increases in the probability of human-mediated incursions of canine rabies may occur in populations subject to management interventions. Therefore, we also modelled the effect on outbreak dynamics of incremental increases in the probability (from the baseline described above) that a dog gained into the study population is exposed. In this scenario, we assume the total number of dogs gained during the study period (and population size) remained the same but the proportion of dogs gained from outside the research site increased by 10%, 20% and 50% from the baseline. This is on the basis that the observed rates of ownership in the study populations were constant during the study period (see Chapter 2) and would probably not change in response to population management. All simulations were truncated at month 34 and any truncated outbreaks (a maximum of 6.4%) were included in the analysis.

Although the entry of free-roaming rabid dogs from communities adjacent to the study populations cannot be excluded, when considering the local terrain surrounding the study areas and the observation that all dogs that moved into the study populations were brought in by people, incursions into the study populations are more likely to be mediated through human translocation of exposed dogs. Therefore, for the purposes of the exploratory analysis, entry of free-roaming rabid dogs into the study populations were ignored. Any transient dip in population size from culling was also ignored.

All analysis were done using R (R Core Team 2014).

5.3 Results

5.3.1 Population characteristics

Although limited to five sites, consistent with other studies, there was a higher density of dogs where there was a higher density of people (Table 5.4). However, the relationship between human and dog population density is not directly comparable between countries. For example, the average dog population densities for Zenzele and Kelusa are similar (~440 km^{-2}) but the human population density in Zenzele (14410 km^{-2}) is four times that of Kelusa (3436 km^{-2}). The proportion of children younger than 7 years of age was higher in Johannesburg

compared to Bali (Zenzele 20.4%, Braamfischerville 15.4%, Kelusa 6.8% and Antiga 10.6%). There was considerable inward and outward movement of dogs by people, with at least one third of the dog population sourced from outside the research sites, and 5-10% of pups and 10-20% of juveniles and adults leaving the research sites (Chapter 2).

Table 5.4 Study population size and density.

	Zenzele	Braamfischerville	Kelusa	Kaler (Antiga)[c]	Ketug (Antiga)[c]
number of households in the study area	2170[a] 2212[b]	2844	203	287	78
mean dog population density (dogs km^{-2})	449.29	225.19	435.61	582.28	130.41
mean number of people per household	4.78	5.27	11.17	6.78	5.86
mean human population size	10375.75	14987.88	2267.51	1945.86	457.08
mean human population density (people km^{-2})	14410.76	11895.14	3435.62	6486.2	725.52
mean human: dog ratio	32.07: 1	52.82: 1	7.89: 1	11.14: 1	5.56: 1

[a] before May 2009; [b] from May 2009 following the erection of an additional street of shacks May 2009; [c] includes data until May 2011

5.3.2 Comparison of outbreak dynamics, R_0, and transmission rates

Outbreak size increased with R_0 and population size (Figure 5.1 and Appendix 5.1). Excluding index cases, for all of the study populations an R_0 of approximately 0.7 generated outbreak distributions with a median outbreak size of 3 and upper 95% quantile of approximately 20 cases, comparable to observational data from Tanzania (Hampson *et al.* 2009). The median outbreak size was similar for all the populations, increasing from three cases for an R_0 of 0.5 to ten cases for an R_0 of 1.2. There was a disproportionate increase in the 95% quantile with R_0 and population size, with a maximum outbreak of ~300 cases in Zenzele (average population size of 323 dogs), corresponding to a maximum outbreak size of 63 cases in Ketug (average population size of 82 dogs) (i.e. for an R_0 of 1.2).

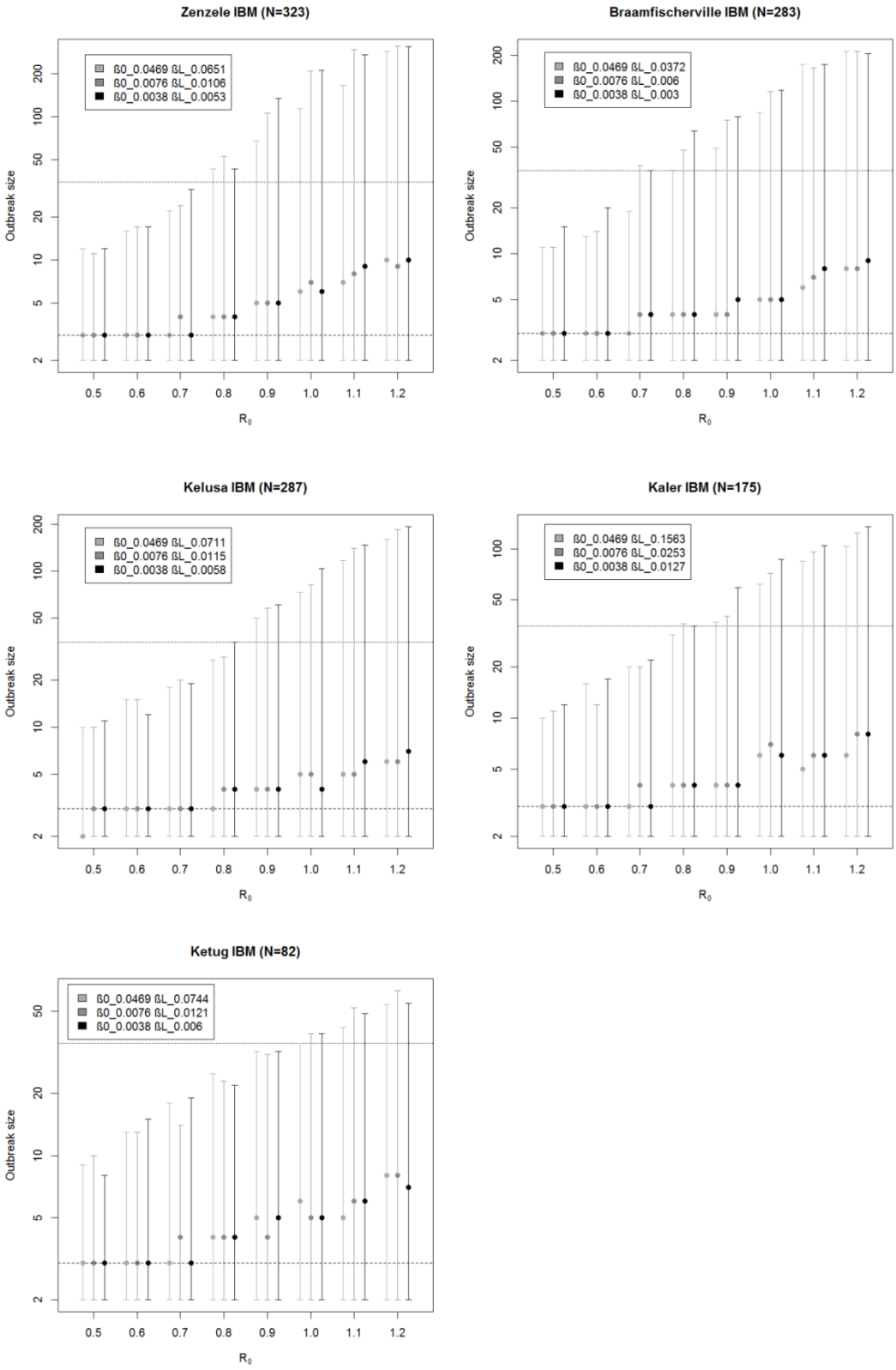

Figure 5.1 Outbreak size for a range of R_0 and transmission rates (β) for the individual-based model (IBM). Each vertical line represents the distribution in outbreak size generated from 1000 simulations. The dot on the vertical line represents the estimated median outbreak size. The top and bottom of the vertical line are equivalent to the estimated 95% and 5% quantile respectively. The top horizontal line indicates the upper limit in outbreak size (of approximately 35 cases) and the bottom horizontal line indicates the median outbreak size (of approximately 3 cases) observed in villages in the Serengeti District (Hampson *et al.* 2009). N = population size.

Median outbreak sizes were comparable for the individual-based and compartmental models; however the 95% quantiles were generally lower for the compartmental models (Figure 5.1 and Appendix 5.1). For a given R_0 there were no substantial differences in outbreak size with transmission and removal rates, although there was a tendency for slightly larger outbreaks with longer infectious periods in Zenzele, Kelusa and Kaler. However, the outbreak dynamics were highly stochastic, with any differences in outbreak size with the transmission and removal rates much smaller than the intrinsic variability in outbreak size. The largest difference occurred in Kelusa for an R_0 of 1.2, with an increase in the 95% quantile from 160 with an average infectious period of 1.4 hours to 192 with an infectious period of 17.4 hours. The probability of an outbreak increased with R_0 but not population size. For an R_0 of 0.7 the probability of an outbreak was ~40% (36-43%), a small fraction of exposed dogs (<6%) did not transition to the infectious state (i.e. were lost through demographic processes), and 54-62% of exposed dogs transitioned to the infectious state but did not instigate an outbreak, rather remained as isolated (index) cases.

5.3.3 Probability of an outbreak with vaccination coverage

The probability of an outbreak following vaccination of a proportion of the population at the start of the time series was similar for the individual-based and compartmental models (Figure 5.2 for a summary of the results for Kelusa and Appendix 5.2 for the results for all of the populations).

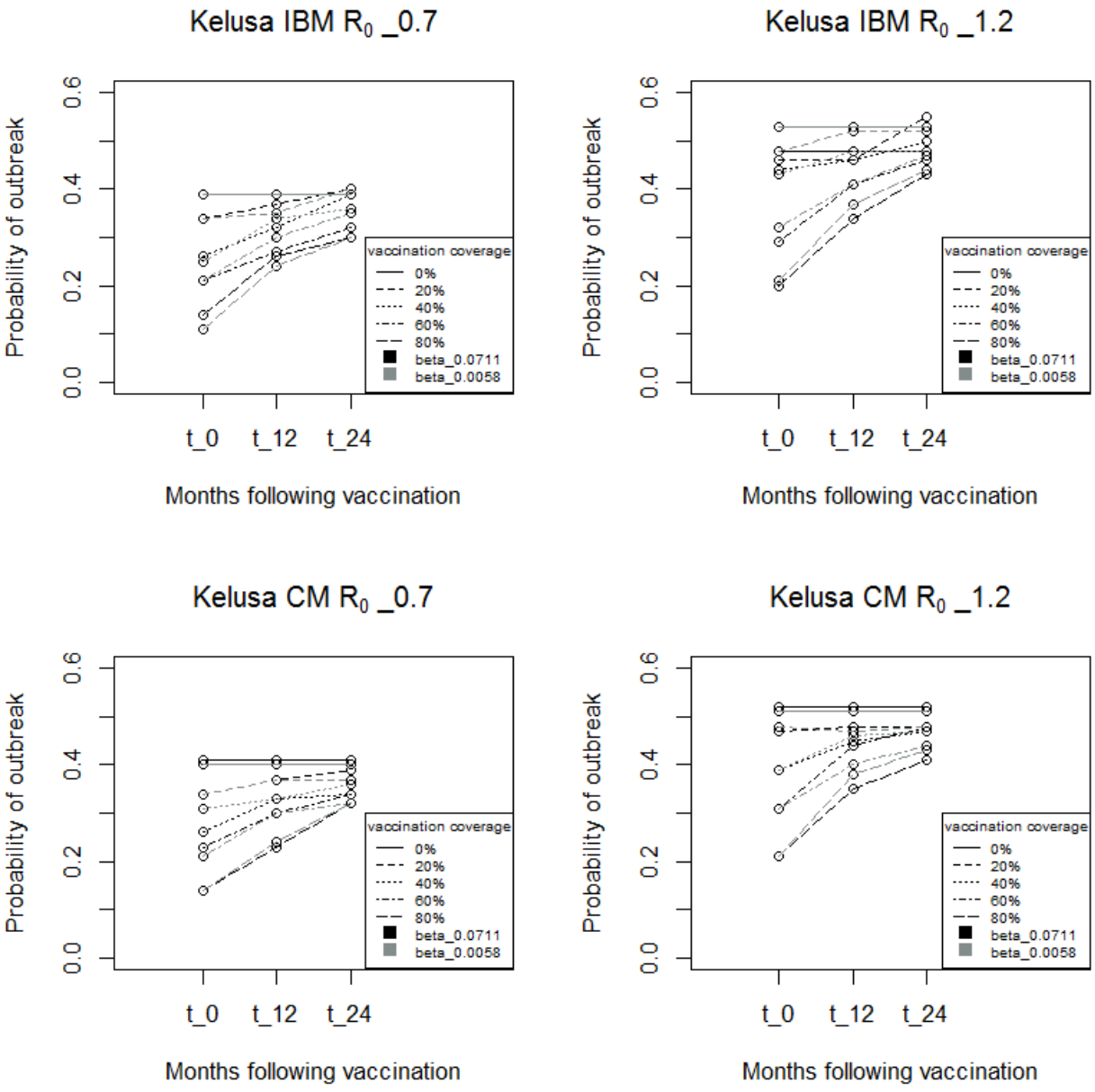

Figure 5.2 Probability of an outbreak in Kelusa at the time of vaccination (t_0) and 12 and 24 months after vaccination for a range of vaccination coverage and transmission rates (beta). The top plots were generated using the individual-based model (IBM) and the bottom plots using the compartmental model (CM).

Vaccination coverage of 80% generally reduced the probability of an outbreak by two thirds immediately following pulse vaccination undertaken at the start of the time series. Although the probability of an outbreak approximately doubled by 12 months after the pulse vaccination (Appendix 5.2). For example, in Kelusa (for an R_0 of 0.7, transmission rate of 0.0058 dogs per km^2 per day and infectious period of 10.1 hours) the probability of an outbreak decreased from 39% prior to vaccination to 11% immediately following 80%

vaccination, but then increased to 24% by 12 months following vaccination. In general, for vaccination coverage of <60% the probability of an outbreak at 24 months was similar to the unvaccinated study population.

Appendix 5.3 shows the estimates of the proportion of the study population still immune 12 and 24 months after vaccination of 60% and 80% of the starting cohort. Declines in vaccination coverage estimated by the SEI models agree with those estimated from the starting cohorts. For example, in Kelusa vaccination coverage declined to 35% in the study population 24 months after a pulse vaccination campaign that achieved 80% coverage. This agrees with the probability of an outbreak of 30% estimated at 24 months after achieving 80% vaccination and vaccination coverage of between 20-40% at the time of vaccination (Appendix 5.2).

5.3.4 Comparison of outbreak dynamics, human-mediated incursions and vaccination coverage

Human-mediated incursions of exposed dogs appear to off-set reductions in the probability of an outbreak achieved through vaccination even with R_0 <1 (Figure 5.3 and Appendices 5.4 and 5.5). For example, in Kelusa (for a transmission rate of 0.0058 dogs per km^2 per day), 80% vaccination coverage at the start of the time series caused a ~25% reduction in the probability of at least one outbreak (from 34% to 26%) as a consequence of normal background (i.e. baseline) movement of dogs during the study period. However, a 20% increase in the probability of human-mediated incursions increased the probability of an outbreak to 33%, comparable to that in unvaccinated populations. For vaccination coverage <80%, a 20% increase in human-mediated incursions increased the probability of an outbreak to above the unvaccinated baseline population. Vaccination coverage <60% at the start of the time series for Kelusa had no effect on the probability of at least one outbreak during the study period or median outbreak size. Overall, median outbreak size reduced slightly (from 3.5 to 2.5) with vaccination coverage ≥60%. Maximum outbreak size reduced substantially with vaccination; in Kelusa by more than two thirds with 80% vaccination coverage (Figure 5.4. and Appendix 5.6).

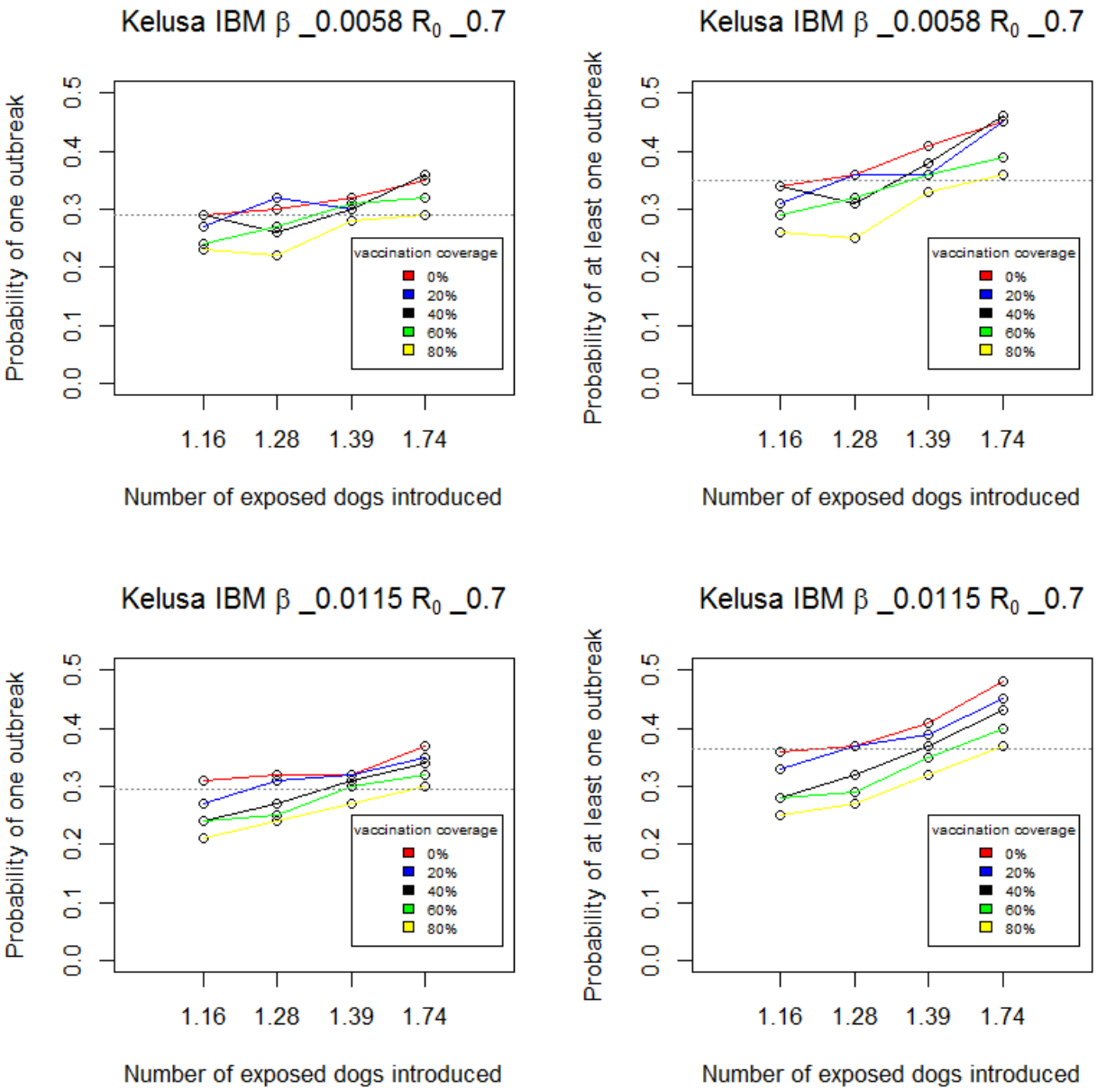

Figure 5.3 Probability of outbreaks with human-mediated incursions for a range of vaccination coverage at the start of the time series and transmission rates (β) in Kelusa. The plots were generated using the individual-based model (IBM). The probability of an outbreak during the 34 month study period is shown on the y-axis. The average number of exposed dogs entering the study population during the study period are shown on the x-axis; and were estimated from empirical data described in section 5.2.3.3. The baseline probability that a dog gained into the study population during the study period was exposed was obtained by dividing 1.16 (number of exposed dogs) by the total number of dogs gained into the study population during the study period (n=478). This probability was included in the model. The number (and the probability) of exposed dogs gained during the study period was then increased incrementally by 10%, 20% and 50% from the baseline as shown. The horizontal line provides a reference to allow for comparisons.

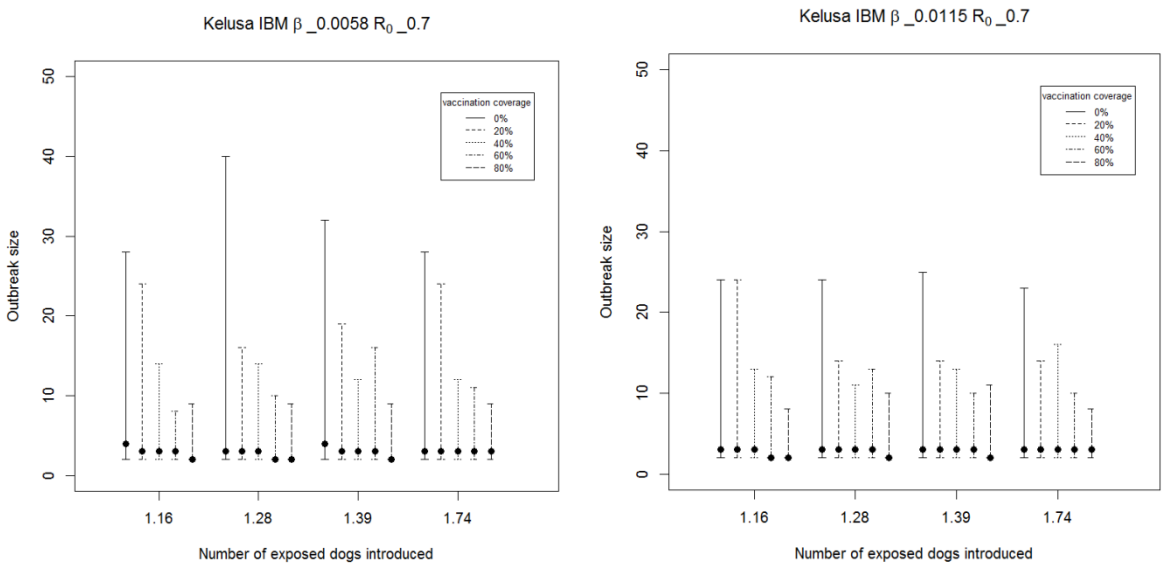

Figure 5.4 Outbreak size with human-mediated incursions for a range of vaccination coverage at the start of the time series and transmission rates (β) in Kelusa (this figure is reproduced in Appendix 5.6 with additional information). The plots were generated using the individual-based model (IBM). The number of exposed dogs gained into the study population during the 34 month study period are shown on the x-axis; see Figure legend 5.3 for the derivation of the number (and probability) of exposed dogs gained into the study population during the study period. Each vertical line represents the distribution in outbreak size generated from 1000 simulations. The dot on the vertical line represents the estimated median outbreak size. The top and bottom of the vertical line are equivalent to the estimated 95% and 5% quantile respectively.

5.4 Discussion

The models used in this study have facilitated exploration of the transmission of rabies in realistic free-roaming dog populations. The structure of the models was underpinned by assumptions regarding the nature of human interference in transmission processes and their application limited through parameterisation from data not from the study populations. Nonetheless, the main outputs, that R_0 at the local (e.g. village) level may be <1 and lower than the global (e.g. provincial level) R_0, and that the local influx of infected dogs by people may off-set the benefits of vaccination, are biologically plausible and important for disease control policy, and hence epidemiological modelling, as discussed below.

To model the hypothesis of human interference in transmission processes, specifically the selective removal of infectious and in-contact dogs scaling with dog population density, we scaled the infectious period, fixing R_0 irrespective of local (i.e. study population) density. This hypothesis is supported by empirical evidence of selective removal (Beran 1982; Ogunkoya, Will & Ezeokoli 1984; Pastoret & Brochier 1998; Belotto *et al.* 2005; Hampson *et*

al. 2009; Tenzin *et al.* 2011; Townsend *et al.* 2013b) and a correlation between dog and human population densities (Oboegbulem & Nwakonobi 1989; Matter *et al.* 1998; Butler & Bingham 2000), also evident in the study populations (Table 5.4). While there are currently no published data regarding scaling of selective removal with density, given the possible importance of human interference in transmission processes, this warrants investigation. In light of this study, this investigation should consider two aspects. The first is how human demography may affect any scaling of selective removal with density. Although there was a higher density of dogs where there was a higher density of people within Johannesburg and Bali, the relationship between human and dog densities is not directly comparable between countries. However, when considering local human demography and culture, the difference between countries in the numbers of people available to selectively remove a rabid dog may be reduced. For example, there was a higher proportion of very young children in Johannesburg compared to Bali. Young children are unlikely to chase and kill rabid dogs, thus reducing the number and density of people likely to selectively remove a rabid dog in Johannesburg. Other factors, such as the proportion of householders at work during the day and the elderly and sick may also be important.

The second aspect that warrants consideration is the degree to which the effective infectious period and transmission rate is reduced through any human interference. For a given R_0 outbreak size was fairly insensitive to the values of the transmission and removal rates in this study. Therefore, from the perspective of modelling outbreak size it is only the value of R_0, and not the particular combination of values selected for these parameters, that is important. However, the values of these parameters have important biological significance that may be important for predicting the impact of different interventions. To model the effect of human-mediated incursions in Kelusa a transmission rate of 4.2 dogs per km^2 per year and an infectious period of ~5 hours was used. These parameters were selected on the assumption that they may reflect what is biologically plausible. The parameter values are consistent with the reported incidence of rabies in Bali (Townsend *et al.* 2013a; Townsend *et al.* 2013b) and reports that community members chased and killed a rabid dog on two separate occasions in the non-study area of Kelusa. Given the rapidity with which the community members were reported to have killed the dogs, it is reasonable to assume that the rabid dogs were unlikely to have been left to roam for an extended period of time. However, the exact duration that the rabid dogs were in the village, presenting a public health risk, is unknown. In this respect, the degree with which any selective removal reduces the effective infectious period justifies further investigation. Finally, any other anthropogenic factors that may scale with human and

dog density and interfere with the contact process, such as traffic and urban infrastructure, should also be considered.

A key prediction from this study is that an R_0 of ~0.7 generates outbreak size distributions consistent with that observed at the village level in the Serengeti, with a median outbreak size <5 cases and an upper limit of approximately 35 cases (Hampson *et al.* 2009). Predictions of a small number of cases at the local level is also consistent with estimates of disease incidence typically <0.5% (Waltner-Toews *et al.* 1990; Windiyaningsih *et al.* 2004; Zinsstag *et al.* 2009; Tenzin *et al.* 2010; Tenzin *et al.* 2011) and the lack of empirical evidence of large fluctuations in dog population density during an outbreak (Hampson *et al.* 2007). This suggests that the R_0 at the local (e.g. village) level is lower than the R_0 estimated for larger geographic areas. This difference may be the result of human-mediated movement of infected dogs, such that exposed dogs are lost from the local population but not from the global population, and the roaming of rabid dogs out of the local population. Together with selective removal and demographic and stochastic processes, the loss of infected dogs from the local population through human-mediated movement may also contribute to reducing the local R_0 <1 with important implications for rabies control, as discussed below. The difference between the local R_0 and global R_0 may also reflect the duration of outbreaks at the global level, which are generally protracted over months or years, compared to small clusters of cases over shorter time frame at the local level.

The implication that human interference may have a substantial effect on local and spatial dynamics should be considered in epidemiological models and disease control policy. Culling is often instigated at the local level, for example by the banjar Head in Bali, as a means to curb a rabies outbreak. However, culling should not be advocated on the grounds that selective removal of diseased animals alone may contribute to the control of rabies (Pastoret & Brochier 1998; Tenzin *et al.* 2011) and because for an R_0 below 1 the probability of an outbreak is low. For an R_0 <1 any outbreak generally only precipitates a short chain of infection even in large populations (Lloyd-Smith *et al.* 2005) and are therefore "self-limiting" irrespective of any culling. However, even though human interference and demographic and stochastic processes may reduce R_0 to <1, reducing the probability of large outbreaks, vaccination still has a protective effect at the local level and is essential. A global R_0 of <2 corresponds to a vaccination threshold of 20-45% necessary to reduce the R_0 to unity, below which deterministic models predict that outbreaks are curbed. Most vaccination programmes are implemented at regional levels and vaccination coverage above this threshold should be maintained through widespread annual vaccination campaigns achieving a vaccination

coverage of at least 70%. When considering stochastic processes, short, rather than long, chains of cases are more likely to occur when $R_0 < 1$ and in smaller populations (Lloyd-Smith *et al.* 2005), which may explain the uniformity in the outbreak distributions for an $R_0 < 1$. These short chains of infection still present a public health risk. As our results demonstrate, vaccination of at least 80% will substantially reduce the probability of an outbreak even in small populations. However, vaccination coverage declines through demographic and immunological processes, necessitating frequent, thorough local vaccination campaigns (Figure 5.2 and Appendix 5.2).

Regular, comprehensive vaccination programmes at the local level are particularly important when considering human-mediated incursions of exposed dogs. Such incursions may limit the efficacy of vaccination even with high coverage (Figure 5.3 and Appendices 5.4 and 5.5). There was considerable inward and outward movement of dogs by people in the study populations (Chapter 2). Therefore, it could be useful to limit baseline movement, by encouraging the local acquisition of dogs, and to ensure that vaccinations are untaken simultaneously with interventions that may increase the influx of infected dogs, such as mass sterilization programmes. Sterilization programmes, implemented at the population-level, are often advocated as a necessary component of canine rabies control (WHO 2004; Reece & Chawla 2006), but are also undertaken to address animal welfare concerns. As with culling, the demand for dogs by communities may result in an increase in dog importation where local supply has been reduced by sterilization; and sterilizations programmes (and culling) may in fact promote the spread of rabies. Therefore, vaccination at the time of sterilization, but also repeatedly following sterilization programmes is strongly recommended. Evaluation of the effect of human behaviour in response to sterilization and culling on dog population dynamics and disease transmission requires far greater consideration in future research.

5.5 Conclusion

We have used simple stochastic epidemic models to explore the local transmission of rabies in realistic free-roaming dog populations. Our modelling of human interference, with regards selective removal and an increase in the movement of infected dogs as an unintended consequence of population management, is currently based on assumption. Community responses to culling and sterilization, in particular their effect on the movement of dogs, is currently being monitored in Kelusa and Antiga, Bali. However, given the critical role that human interference may play in the transmission of canine rabies, this warrants more extensive research.

Chapter 6

General discussion

Canine-rabies can be effectively controlled through vaccination (Cleaveland *et al.* 2003; Schneider *et al.* 2005; Cleaveland *et al.* 2006; WHO 2013), however the impact of vaccinations on disease incidence may be affected by dynamic demographic and immunological processes, particularly through their effect on vaccination coverage and rabies transmission. To improve rabies control, through field interventions and epidemiological modelling, the research presented in this thesis used a multifaceted approach to explore the effect of demographic and immunological processes, and their regulatory factors, on population and disease dynamics and vaccinal responses in two free-roaming dog populations in Johannesburg, South Africa, and two in Bali, Indonesia.

The main conclusions from this work are:

1. Despite complex population dynamics, and geographical and cultural diversity, the ecology of the dog populations was similar.

2. Human factors are likely to be critically important in the transmission and control of canine-mediated rabies.

3. Many popular perceptions regarding the ecology of free-roaming dogs and the control of canine rabies may be wrong.

4. Widespread, sustained vaccination programmes are essential in rabies endemic areas.

There are several important similarities between the study populations that affect canine rabies transmission and control and population control. These similarities are primarily a function of people and their close relationship with domestic dogs; this relationship is universally recognised. In contrast to other species affected by rabies, human behaviours may have important positive and negative effects on population and disease dynamics in domesticated dogs, which in turn impacts on planning rabies control programmes; and, this is the first study to properly identify this.

Almost all of the identified dogs in the study population were owned and fed regularly by their owners, with important implications for vaccine delivery during vaccination campaigns.

Owners generally facilitate vaccination of their dogs (Lembo *et al.* 2010; Knobel *et al.* 2013) through identification and restraint of their animal. Unowned dogs may be more difficult to access for vaccination, potentially reducing effective vaccination coverage (Hampson *et al.* 2009), particularly if the fraction of unowned dogs is large. At least 70% of the dog population was vaccinated through door-to-door vaccine delivery and with the assistance of owners, even in Bali where the majority of the dogs needed to be caught by net. In Bali community engagement, including a simple ad hoc pictorial pamphlet delivered door-to-door, raised awareness of rabies and explained what a vaccine is and the reason for vaccinating dogs in order to protect people from rabies. The pamphlet was designed to cater for the illiterate and poorly educated. As a result, even though most owners could not restrain their dogs manually, many managed to confine their dogs to the kitchen to facilitate capture by net. This reduced the time and effort required to catch the dogs. Furthermore, all identified dogs resident in the study population belonged to households in the study area, therefore vaccination coverage was accurately measured during vaccine delivery.

The mode of ownership of the dogs in the study population is consistent with increasing evidence that, globally, most free-roaming dogs are owned (WHO & WSPA 1990; Cleaveland & Dye 1995; Butler & Bingham 2000; Estrada *et al.* 2001; Windiyaningsih *et al.* 2004; Gsell *et al.* 2012) and fed regularly by their owner (Brooks 1990; de Balogh, Wandeler & Meslin 1993; Butler & Bingham 2000). However, historically it was assumed that the free-roaming dogs in the four communities were unowned or owned but neglected, whereby the dogs primarily scavenged for food to survive. This initial uncertainty regarding ownership status parallels conflicting reports in the literature regarding the mode of ownership and food sources of free-roaming dogs. Implicit in many canine epidemiological and ecological models is the assumption that dog populations are regulated through environmental resource constraints on births and deaths (Wandeler 1985; WHO & WSPA 1990; Kitala *et al.* 2002; Vandermeer & Goldberg 2003; Hampson *et al.* 2007; Zinsstag *et al.* 2009; Totton *et al.* 2010). This implies that all individuals in the population are unowned and compete for a common resource to survive, most likely environmental refuse, consistent with limited observations (Pal 2001). By contrast, other estimates of the unowned fraction of the population are variable, ranging from 0%, including in sub-Saharan Africa (Butler & Bingham 2000) and South East Asia (Estrada *et al.* 2001; Windiyaningsih *et al.* 2004), to 20-30% (Vos & Turan 1998; Matter *et al.* 2000; Durr *et al.* 2009). A further complication is that a fraction of owned dogs may be neglected and left to scavenge a variable proportion of their diet (Ortega-Pacheco *et al.* 2007a; Ratsitorahina *et al.* 2007), or that a fraction of the

population “belongs” to the community and source variable proportions of their diet from refuse and community members but not a specific owner (Reece & Chawla 2006; Reece 2007). Although the health status of unowned or owned neglected dogs is not reported, it is a widely-held perception that they are in sufficiently good condition to be sustained without direct human oversight. This variability in mode of ownership may reflect true differences in the ecology of dogs globally. Alternatively, it may be a consequence of extrapolations of ecological principles from wildlife to a domesticated species, classification of dogs as unowned if owners are not immediately apparent, or a consequence of the practical difficulties definitively identifying unowned dogs in reasonable or good body condition, particularly where most dogs are owned.

Definitive identification of the fraction of unowned, healthy dogs in the study population would have required intensive, individual-level surveillance of dogs identified as not belonging to households in the study area and quantification of variations in the availability, nutritional content and uptake of environmental resources; neither of which were practicable within the scope of this study. Measures of food provided by owners were also constrained through illiteracy and poor education and the inability to validate the measurements. Indirect assessment of population-level ownership, i.e. population regulation through environmental resource constraints, was hampered by the lack of variation in population size (or density) and births and deaths. Mark-recapture methods used previously to identify the unowned fraction of a population (Fishbein *et al.* 1992; Matter & Fico 1998; Matter *et al.* 1998; Vos & Turan 1998; Matter *et al.* 2000; Cleaveland *et al.* 2003; Kayali *et al.* 2003b; Durr *et al.* 2009; Kaare *et al.* 2009; Gsell *et al.* 2012) may have been limited by measurement error and statistical variations, violations of model assumptions and, more importantly, practical limitations of repeat measures using photographic mark-recapture to identify a resident population. Therefore, a multifaceted approach, combining all practicable methods, was used to assess mode of ownership and food sources of the dogs in the study population. Methods included repeat direct observations of identified dogs, household surveys and subjective assessment of environmental refuse, community-based participatory methods (Chambers 1994a; Chambers 2007) which took advantage of the close relationship between dogs and people and local knowledge of the enumerators as well as the community, and assessment of the relationship between body condition and energy requirements.

From this approach, there was no evidence that population size was regulated through environmental resource constraints, nor was there any evidence that a fraction of the healthy population was unowned or owned but neglected. Rather, population size was regulated

through the demand for dogs. All dogs resident in the study population that were in reasonable or good body condition belonged to households in the study area and were fed regularly by the household. Most of the small number of dogs that did not belong to households in the study area were emaciated. Although it was not verified whether these dogs were owned outside the study area, their condition was consistent with community perceptions that they were unowned, not fed by non-specific community members, and that environmental refuse probably provided inadequate nutrition. A common assertion is that free-roaming dogs in impoverished communities are unowned or owned but neglected. This was not the case in the study populations; most householders provided adequate care and nutrition for their dogs despite their poverty. In addition to vaccine delivery, these observations have wide-reaching implications for animal welfare. Had a population of unowned, healthy dogs been identified it may absolve humans from the responsibility of providing adequate care for a domesticated species.

Several other important commonalities between the four communities impact the planning of rabies control, particularly epidemiological modelling and vaccine delivery. No population growth, or a progressive decline in population size, occurred during the study period, consistent with similar rates of (susceptible) dogs gained into (means of 10-19) and (potentially vaccinated) dogs lost from (means of 11-20) the study population each month (Appendices 2.10 and 2.11). Reliable estimates of these demographic processes, including growth rates, are required for dynamical models, including compartmental models of local transmission (Cleaveland & Dye 1995; Kitala *et al.* 2002; Hampson *et al.* 2007; Zinsstag *et al.* 2009; Totton *et al.* 2010). As previously discussed, population size, and the acquisition of (susceptible) dogs, is regulated through human demand for dogs. Previous estimates of growth have generally been at the national (Brooks 1990) or district (Butler & Bingham 2000; Kitala *et al.* 2001; Hampson *et al.* 2009) level and may reflect the ecological heterogeneity within, and limited movement of dogs into and out of, large geographical areas. This study has generated reliable estimates of demographic parameters and has demonstrated that growth may be limited in established communities where geographical expansion is minimal.

A common perception is that, in communities where the majority of dogs are free-roaming, there are “too many dogs”. Contrary to expectations, household rates of ownership were consistently low, with only a fraction of the households owning dogs (approximately 10% in Johannesburg, 70% in Kelusa and 40-50% in Antiga) and an average of 1.3-1.7 dogs per dog owning household, comparable to the rates of dog ownership in the UK (Murray *et al.* 2010).

Low rates of ownership may support vaccine delivery, such that fewer dogs need to be vaccinated to achieve the target vaccination coverage of at least 70% during annual vaccination campaigns (Coleman & Dye 1996; Cleaveland *et al.* 2003; Hampson *et al.* 2009; WHO 2013). Furthermore, a substantial fraction (20-40%) of the population was present for the entire study period. A large proportion of mature adults may live longer than previously recognised (Oboegbulem & Nwakonobi 1989; Brooks 1990; Rautenbach, Boomker & de Villiers 1991; Margawani & Robertson 1995; Robinson *et al.* 1996; Butler & Bingham 2000; Kitala *et al.* 2001). While every available dog should be vaccinated against rabies irrespective of age class, vaccinating adults may be particularly important in maintaining vaccination coverage. Vaccination coverage in the study population was estimated to remain within the critical threshold of 20-45% necessary to curb an outbreak (Hampson *et al.* 2009) two years after a pulse campaign that achieved 60% coverage (assuming that all vaccinated individuals were protected from challenge). This deterministic threshold is consistent with estimates of a global R_0 of <2. Most control programmes are implemented at the regional level and vaccination coverage above this threshold should be maintained through widespread, annual vaccination campaigns achieving a vaccination coverage of at least 70%. However, dynamical models from this study, that account for human interference (i.e. selective removal, or the swift identification and killing of infectious and in-contact dogs by local communities, and the translocation of dogs by people) in transmission, predict an R_0 of <1 in local populations (e.g. a Bali village) with outbreaks generally limited to short chains or clusters of cases consistent with epidemic theory (Lloyd-Smith *et al.* 2005). These short chains of infection present a significant public health risk. In light of these predictions, previous interpretations (in Chapters 1, 2 and 4) of the critical threshold (reducing R_0 to unity) in local populations may be insufficient to prevent human disease. However, high levels of vaccination coverage is still predicted to substantially reduce the probability of a cluster of cases even in small populations. Thus, the recommended vaccination coverage of 70% (WHO 2013) should also be achieved at the local level.

Vaccination coverage declines in populations through time through demographic and immunological processes. Although almost all of the dogs in Johannesburg, and presumably Bali, seroconverted (virus neutralizing antibody titre ≥0.5 IU/ml) to high-quality, inactivated cell-culture rabies vaccine irrespective of health status, titres were predicted to decline to near negligible levels (<0.1 IU/ml) within two years for a proportion of the vaccinated population. There may be an increased susceptibility to natural exposure in dogs with low titres (Precausta *et al.* 1985; Aubert 1992; Bahloul *et al.* 2006; CDC 2008; Siegrist 2008), but also

in the remaining unvaccinated dogs in the population given the decline in effective vaccination coverage and herd immunity. This reinforces the importance of frequent and thorough vaccination campaigns.

A substantial fraction of the study population was sourced from outside the research sites by the dog owners. Translocation of dogs may contribute to the spatial spread of rabies between populations (Denduangboripant *et al.* 2005; Coetzee & Nel 2007; Kasempimolporn, Jitapunkul & Sitprija 2008; Beyer *et al.* 2010; Talbi *et al.* 2010; Townsend *et al.* 2013b) and incursions of rabies into local populations (Chomel *et al.* 1987; Beran & Frith 1988; Robinson *et al.* 1996; Zinsstag *et al.* 2009). Human-mediated incursions of exposed dogs may off-set reductions in disease incidence, R_0, and vaccination threshold through human interference and demographic and stochastic processes, thus necessitating widespread and sustained vaccination campaigns.

Complex population dynamics may also have practical consequences in terms of population control. Sterilization programmes, implemented at the population level, has been advocated as a necessary component of canine rabies control (WHO 2004; WHO 2013). These programmes are associated with a reduction in the incidence of rabies when sterilized dogs are vaccinated simultaneously (Reece & Chawla 2006), however it is uncertain to what extent sterilization itself is necessary for rabies control. Sterilization programmes may gradually stabilise or reduce population size (or density) over several years (Reece & Chawla 2006; Totton 2009; Totton *et al.* 2010). Sterilizations are also hypothesized to extend vaccination coverage by increasing the longevity of dogs and reducing the number of new, susceptible dogs entering the population through reducing local births. Although reductions in population density are unlikely to reduce the transmission of rabies (Coleman & Dye 1996; Kitala *et al.* 2002; Hampson *et al.* 2009), they may reduce the number of dogs that require vaccination. However, mass sterilizations may have a reduced effect where population growth is already limited and a large proportion of the population originates from outside the area. Furthermore, reducing the number of dogs that require vaccination may be unnecessary when rates of dog ownership are already low. From the available data, the effect of sterilizations on population structure (Totton 2009; Totton *et al.* 2010) and life expectancy is unclear. Increasing the adult fraction may only have a minor impact on vaccination coverage if the fraction prior to the intervention was already large (Totton *et al.* 2010). Most critically, the demand for dogs by communities may result in an increase in dog importation (some of them infected with rabies) where the local supply has been reduced by sterilization (or culling discussed below). Dynamical models from this study indicate that small (i.e. 20%) increases

in human-mediated incursions may off-set the advantages of high levels of vaccination, particularly the probability of an outbreak. Therefore, evaluation of the effect of human behaviour in response to sterilization (and culling) on dog population dynamics and disease transmission needs far more research attention.

Sterilizations are also advocated to reduce the number of puppies and juveniles in a population on the basis that a proportionally higher incidence of rabies is often reported in these age classes (Belcher, Wurapa & Atuora 1976; Malaga, Lopez Nieto & Gambirazio 1979; Beran 1991; Mitmoonpitak, Tepsumethanon & Wilde 1998; Widdowson *et al.* 2002); with a serious risk to the public given the close relationship between humans and puppies (Mitmoonpitak, Wilde & Tepsumethanon 1997; Taiwo *et al.* 1998; WHO 1998; Widdowson *et al.* 2002; Awoyomi, Adeyemi & Awoyomi 2007). The higher incidence of rabies in young dogs may be the effect of low vaccination coverage in this age class, given that puppies are often excluded from vaccination programmes (Chomel *et al.* 1987; Beran & Frith 1988; Brooks 1990; Matter & Fico 1998; Mitmoonpitak, Tepsumethanon & Wilde 1998; Matter *et al.* 2000; Gunatilake, Wimalaratne & Perera 2003; Flores-Ibarra & Estrella-Valenzuela 2004; Kongkaew *et al.* 2004; Awoyomi, Adeyemi & Awoyomi 2007; Durr *et al.* 2009; Kaare *et al.* 2009; Touihri *et al.* 2011; Davlin *et al.* 2013) and population immunity declines following a vaccination campaign. Dogs less than 12-13 weeks of age are generally excluded from vaccination programmes on the assumption that they have maternal antibodies and immature immune systems which may limit the immune response to rabies vaccine. Evaluation of the effect of maternal antibodies and immune function of puppies on rabies vaccine-induced immune responses is limited. Maternal antibody may interfere with immune responses (Day & Schultz 2011; Siegrist 2012; Tizard 2013), particularly in puppies 8 weeks of age or younger vaccinated with modified live vaccine under field conditions (Aghomo, Oduye & Rupprecht 1990). However, at least under experimental conditions, maternal antibodies and immune function may not limit the immune response to inactivated vaccines which stimulate both B- and T- cell responses (Siegrist 2012), as demonstrated in puppies vaccinated with Rabisin at 2 weeks of age (Chappius 1998). The results from this study support these prior observations (Appendix 6.1). Therefore, rather than try to reduce the proportion of puppies in the population through sterilizations, it may be more efficient to simply vaccinate them with commercially available, high quality, inactivated vaccine.

Culling is commonly instigated at the local (e.g. banjar or sub-village) level in response to rabies cases. Similar to sterilization programmes (discussed above), culling assumes that encounters between susceptible and infectious individuals and, thus, disease incidence

increases with host density (i.e. is density-dependent). Culling reduces host density in order to reduce the incidence of the disease, and reduce host density to below the deterministic threshold for invasion ($R_0 = 1$) to halt the spread of an epidemic (chapter 1). Although the role of density (and other factors) in the transmission of rabies is currently poorly understood, the available evidence suggests that there is no clear relationship between dog population density and transmission (Coleman & Dye 1996; Kitala *et al.* 2002; Hampson *et al.* 2009), and reductions in density through the mass killing of predominately healthy dogs (or through sterilizations) are unlikely to reduce the transmission of rabies. While it cannot be ruled out that rabies transmission is independent of host density (i.e. is frequency-dependent), consistent with the low incidence of canine rabies (generally <0.5%) (Waltner-Toews *et al.* 1990; Windiyaningsih *et al.* 2004; Rothman, Greenland & Lash 2008; Zinsstag *et al.* 2009; Tenzin *et al.* 2010; Putra *et al.* 2011; Tenzin *et al.* 2011; Townsend *et al.* 2013a; Townsend *et al.* 2013b) a more plausible explanation is that selective removal "disrupts" density-dependent transmission. Selective removal reduces the effective infectious period (Hampson *et al.* 2009) and may reduce the incidence of rabies (Pastoret & Brochier 1998; Tenzin *et al.* 2011). Should selective removal scale with human, and thus dog, density as hypothesized (Chapter 1), then the effective infectious period will scale inversely with host density and transmission will appear to be independent of host density.

Selective removal presents an alternative mechanism to density reduction to reduce R_0 to below unity. Indeed, euthanasia of dogs showing clinical signs is advocated to control rabies (WHO 2013). As noted above, dynamical models from this study, that account for selective removal and stochastic and demographic processes, predict an $R_0 < 1$ in local populations, with a low probability of an outbreak. Any outbreaks that do occur are generally small and, thus, "self-limiting" irrespective of culling. Furthermore, reducing host density to below the deterministic threshold may not achievable. Even in Ketug, the smallest of the research populations with a density of ~130 dogs per km^2 and assuming the lowest transmission rate used in the dynamical models (of 0.0038 dogs per km^2 per day), density would have to be almost halved to reduce R_0 to unity. Similar to sterilizations, although culling may reduce the number (but not proportion) of dogs requiring vaccination (Rosatte *et al.* 2007), density reduction may be off-set by the continual influx of dogs brought into the population by people. This influx may be exacerbated in response to culling, with a corresponding increase in the incursions of rabies especially into larger populations. However, more importantly, culling is ethically questionable given that most free-roaming dogs are probably owned and

accessible for vaccination, and vaccination has proven efficacy (Cleaveland *et al.* 2003; Schneider *et al.* 2005; Cleaveland *et al.* 2006; WHO 2013).

One important difference between the communities was in the handleability of the dogs for vaccination. The majority of the dogs in Johannesburg were amenable to gentle restraint, with a soft muzzle and leash, for vaccination and blood sampling. In contrast, in Bali the majority of the dogs could not be safely restrained without a net. Although the reasons for this difference are unclear, it may be attributable to subtle cultural differences in human-dog interactions. In particular, preliminary data from the follow-up study and informal discussion with community members indicate that puppies are more likely to be tied up in Bali during the sensitive period critical for social development (Serpell & Jagoe 1995; Appleby, Bradshaw & Casey 2002). Restraint by net is more stressful to the dog, time consuming and costly, making vaccine delivery more difficult, potentially deterring those responsible for rabies control to undertake regular and thorough vaccination campaigns.

Implications of this research

The findings from this research have important implications for rabies control, particularly given the similarities between the four communities in this study and other communities where most free-roaming dogs are owned and fed by their owners.

In contrast to other species affected by rabies, the complex relationship between dogs and people is critically important in the transmission and control of canine-mediated rabies and should not be ignored. Community involvement is essential for vaccine delivery and also to access essential information for rabies and population control. It is often assumed that free-roaming dogs are unowned, effectively wildlife, or that the dogs are neglected by owners and that community members are disinterested in their welfare. Rather, the starting point should be that most, if not all, free-roaming dogs in a community are owned and wanted, particularly if they are in reasonable or good body condition, most are accessible for vaccination through their owner, and that the community can provide essential information regarding the dogs in their community.

Human factors should also be considered with regards the level or scale of interventions. Human-mediated movement of dogs may contribute to the spatial spread and incursions of rabies, therefore vaccination programmes need to be spatially ambitious and sustained. Vaccination coverage at regional levels should be maintained above the deterministic threshold of 20-45%, thus vaccination campaigns should achieve a uniform, widespread

coverage of 70% (Townsend *et al.* 2013b; WHO 2013). Importantly, while the deterministic threshold of 20-45% necessary to curb an outbreak may not be applicable at the local level, dynamical models from this study predict that regularly vaccinating 60-80% of the local population substantially reduces the probability and size of chains of infection. Given declines in vaccination coverage following campaigns through demographic and (probably) immunological processes, and predicted rebound in the probability of an outbreak following vaccination, vaccination campaigns should be repeated annually consistent with World Health Organization recommendations (WHO 2013).

The level or scale of interventions intended to reduce population density (or size) should also be considered. Where mass sterilization programmes are implemented over a large geographical area, such as at the city level (Reece & Chawla 2006; Totton *et al.* 2010), the acquisition of dogs from outside the area may have a limited effect on declines in population density on the basis that the influx of dogs may be relatively small compared to number born in the population. Whereas, consistent with this study, a substantial proportion of the population may originate from outside smaller communities or more localised areas, off-setting any reductions in density through sterilization at this level. Indeed, simple assumptions that populations are closed and most dogs originate locally may not be valid, especially in smaller populations. Reductions in the number of puppies born locally may be off-set by an influx of dogs from outside the area. Sterilization programmes are undertaken for a variety of reasons (Jackson 2013). These programmes are generally expensive and labour intensive (Morters pers comm), therefore it is important that the aims of the programme are clearly defined and the limitations of the programme understood. For example, targeted sterilization, i.e. determined as appropriate for each dog individually, may positively impact animal welfare and nuisance behaviour (Reece, Chawla & Hiby 2013) but may have limited impacts on population density.

Although it may be counterintuitive, it should not be assumed that fewer dogs in a population (achieved through density reduction, especially culling) equates to less rabies. There is still considerable uncertainty regarding the factors that drive rabies transmission, particularly the role of host density, however culling is not recommended as a means to control canine rabies (Chapter 1) (WHO 2013). Rather, rabies should be controlled through regular, widespread vaccination with high quality, inactivated vaccine, that achieves at least 70% coverage (WHO 2013), preferably in conjunction with community engagement.

The effect of human behaviour on rabies transmission, specifically human-mediated movement of exposed dogs, has been factored into spatial models of canine rabies (Beyer *et al.* 2010; Townsend *et al.* 2013b). However, human behaviour should also be considered for models of local disease dynamics. Amendments to these models may include no population growth or decline in population size, exclusion of density-dependent demographic processes specific to population regulation through environmental resource constraints, and demographic parameters that account for selective removal and human-mediated movement of dogs.

Further studies required

Priorities for future work include:

1. Determining the factors responsible for the differences in the handleability of dogs may reduce the need for restraint by net for vaccination, lessening the cost and time for vaccination delivery and improving animal welfare. Proper evaluation of human-animal interactions by an anthrozoologist and other factors, such as genetic differences in temperament, is warranted. To balance public health risks, simultaneous evaluation of the risk of infection of owners from zoonotic pathogens, such as dermatophytes and rabies, through increased handling of their dogs (WHO 1998; Widdowson *et al.* 2002) should be undertaken.

2. Assessment of human behaviour in response to culling and sterilization is currently underway in Kelusa and Antiga, Bali. Specifically, these interventions may increase the translocation of dogs, potentially introducing rabies into the community. Culling and mass sterilization programmes are carried out world-wide in response to rabies outbreaks, but also for population management. This study showed a substantial fraction of dogs is sourced from outside the village independent of culling and sterilization. Should these interventions drive an increase in human-mediated movement of dogs sufficient to increase the local incidence of rabies, this adds to the importance of controlling rabies by vaccinations and not density reduction. Where sterilization programmes are established, then it may be mandatory that all dogs in the location of the programme (whether they are sterilized or not) are vaccinated against rabies.

3. Evaluating the factors that determine the rates of ownership may be important for disease and population control, particularly the effect of rabies outbreaks on the acquisition and

disposal of dogs. Density reduction, through culling or mass sterilization programmes, is often undertaken in response to rabies outbreaks. However, the impact of these interventions on disease control may be further limited when declines in population size (or density) are already driven by the community, possibly through the fear of rabies or liability arising from dogs bites to people in rabies-endemic areas. Although the reasons for the low rate of ownership in the study areas are unclear, informal discussions with community members suggest that poverty, i.e. being able to afford to feed a dog, and work commitments are causal factors. If this is indeed the case, it reinforces the points that community members generally consider the provision of adequate care for their dogs a priority and the importance of community engagement in disease and population control.

4. Exploring evidence for selective removal of diseased dogs scaling with dog population density might improve dynamical models of local transmission, however obtaining such evidence may be unrealistic. The distinctive presentation of rabies has facilitated detailed contact tracing, which has contributed significantly to understanding local and global transmission dynamics and the effectiveness of vaccination strategies (Hampson *et al.* 2009). While collecting similar data across geographic locations with different dog densities may provide insights into local transmission dynamics it may prove impractical.

5. Evidence for resident populations of healthy, unowned dogs would have wide-reaching implications for rabies control and animal welfare. However, robust evidence of this sub-group would require intensive monitoring, possibly with GPS collars, for ownership and variations in food sources and health status of at least a small number of dogs identified by locals as not belonging to a reference household. Although there is increasing evidence that these dogs are unlikely to exist, given the heterogeneity in communities (dogs and people) globally, investigation is warranted, particularly India where edible refuse is reported to be a food source for free-roaming dogs (Pal 2001; Reece 2007). Nonetheless, reliable identification of unowned, healthy dogs may be impractical.

6. Evidence of Lagos Bat-like Virus in Bali, suggesting spill-over of a Lyssavirus from bats to dogs, presents a serious public health concern that justifies further investigation (Hayman *et al.* 2013; Peel *et al.* 2013). Exposure of the public to Lyssaviruses other than RBV is of particular concern in light of anecdotal reports of a bat-cave temple priest

dying from encephalitis unrelated to exposure to a dog and two dogs vaccinated with Rabisin at least twice over several months dying from rabies.

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Appendix 2

Appendix 2.1 Questionnaire development and implementation.

The questionnaires were piloted in a sub-set of households in a non-study area of Braamfischerville (n=~15). Questionnaires were subsequently modified for use in Bali through meetings with the enumerators in April 2008. The same questionnaire was used for each census and revisit in all four study areas, with the exception of a few culturally specific questions and, from the second census onwards, the addition of owner reported clinical signs for the previous three months. Specific questions related to either the previous seven days or the previous three months, i.e. since the last survey. Responses were categorical, for example: *When did you last feed this dog? – today / yesterday /other (specify) /don't know*; or open, for example *When did you get this dog?* The format of the question *Why did you not get a dog from inside the site (names)?* was changed from open ended to categorical in November 2009 because of cultural difficulties interpreting the question. In Bali, the questionnaire was bilingual (English and Bahasa with the Bahasa back-translated). Several languages are spoken in Johannesburg, including English, therefore the questionnaire was written in English and the accuracy of the various translations checked regularly with the multi-lingual enumerators throughout the study period. The respondent was the person/s in the household that the householders collectively identified as most knowledgeable about the dog, which was not necessarily the owner. Respondents under 16 years of age were always interviewed with an adult present. The same respondent was generally interviewed at each time point. When a respondent was not available (primarily in the Johannesburg sites), the household was revisited at least once during the same survey period to locate a respondent.

Appendix 2.2 Household characteristics.

	Zenzele	Braamfischerville	Kelusa	Antiga
number of households in the study area	2170[a] / 2212[b]	2844	203	365
total number of registered dogs	1022	882	707	600[c] / 629[d]
number of dog-owning households at first time point	261 (12.0%)	212 (7.5%)	146 (71.9%)	179 (49.0%)
number of dog-owning households at the last time point	222 (10.0%)	222 (7.8%)	148 (72.9%)	153 (41.9%)[c] / 152 (41.6%)[d]
number of dogs per dog-owning household at first time point	1 dog: 202	1 dog: 165	1 dog: 76	1 dog: 130
	2 dogs: 43	2 dogs: 35	2 dogs: 47	2 dogs: 36
	3 dogs: 11	3 dogs: 10	3 dogs: 15	3 dogs: 8
	4+ dogs: 5	4 dogs: 2	4+ dogs: 8	4+ dogs: 5
number of dogs per dog-owning household at the last time-point	1 dog: 170	1 dog: 175	1 dog: 76	1 dog: 112[c] / 107[d]
	2 dogs: 38	2 dogs: 39	2 dogs: 49	2 dogs: 32 / 34
	3 dogs: 12	3 dogs: 4	3 dogs: 20	3 dogs: 7 / 10
	4 dogs: 2	4+ dogs: 4	4+ dogs: 3	4 dogs: 2 / 1

[a] before May 2009; [b] from May 2009 following the erection of an additional street of shacks May 2009; [c] until December 2010; [d] until May 2011;
the estimated mean human population density (km^{-2}) for each study area: Zenzele 14410.76, Braamfischerville 11895.14,
Kelusa 3435.62, Antiga 2558.95 (Kelod / Kaler 6486.20 and Ketug 725.52)

Appendix 2.3 and 2.4 Sex ratios of the registered population (see tables below).

The marked skew towards males in Kelusa and Antiga and the disparity between litter size determined during routine surgical sterilization of dogs from other villages in Bali and reported litter sizes for the study populations is probably a function of under-reporting of dumped neonates, particularly females. Although not systematically reported, at least 12% and 44% of neonates were killed, i.e. typically drowned in rice fields or the river, in Kelusa and Antiga respectively. In Bali the majority of dogs were owned for security and males were perceived to be better guard dogs than females; whereas in Johannesburg dogs were owned for a variety of reasons, including companionship and security.

Appendix 2.3 Sex ratios of the registered populations (males: females)[b].

	first time point	last time point
Zenzele	0.54: 0.46	0.53: 0.46
Braamfischerville	0.52: 0.48	0.58: 0.42
Kelusa	0.75: 0.25	0.75: 25
Antiga	0.77: 0.23	0.81: 0.19[a]

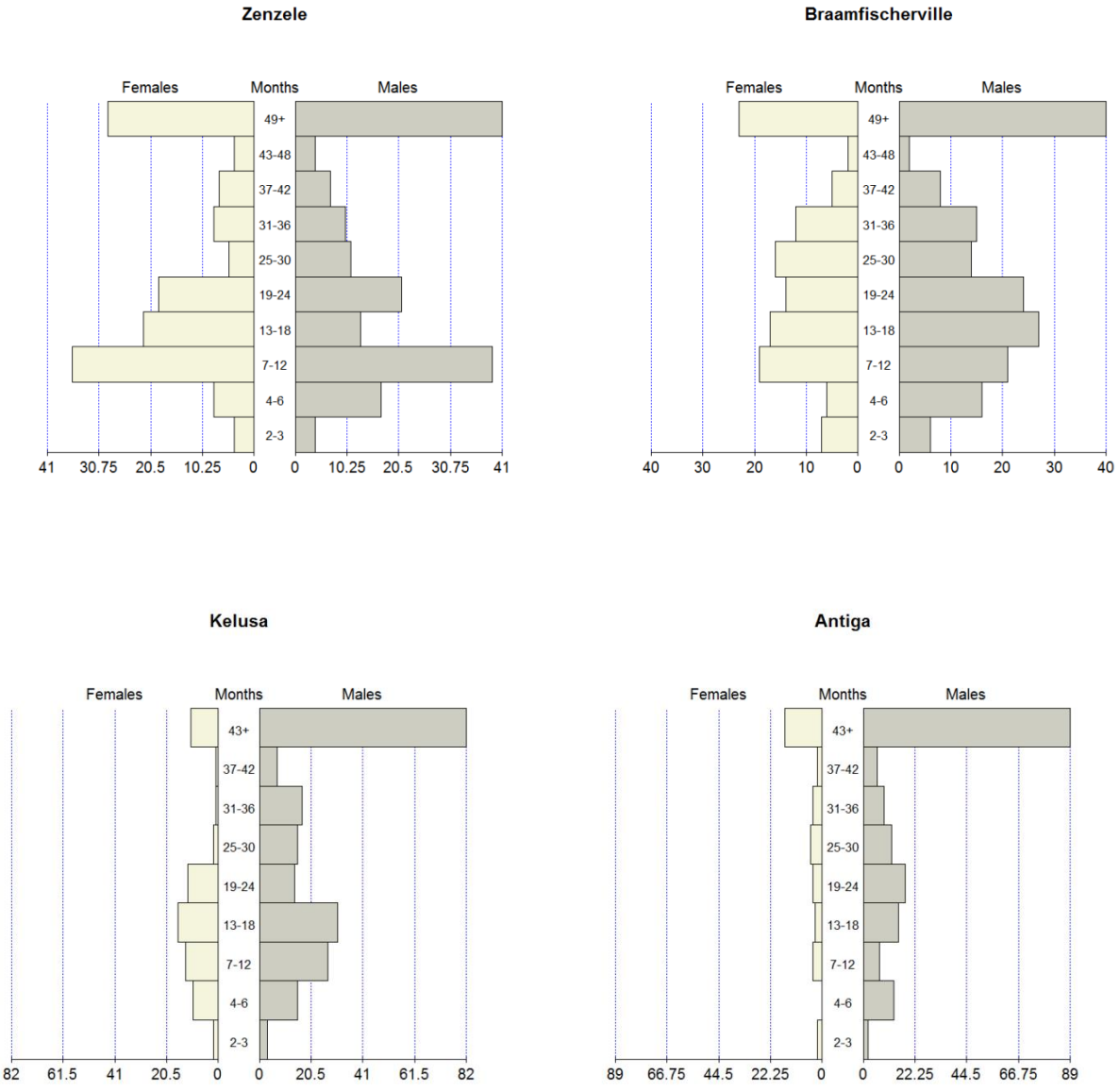
[a] including 2011 data; [b] excludes missing information

Appendix 2.4 Owner reported litter sizes for the study populations, and litter sizes determined during sterilization of pregnant bitches from similar populations elsewhere in Johannesburg and Bali during the study period.

	number of litters	mean	median	minimum	maximum
litter size at sterilization **Johannesburg**	22	7.0	6	4	11
reported litter size **Zenzele**	273	5.7	6	1	13
reported litter size **Braamfischerville**	117	6.0	6	1	12
litter size at sterilization **Bali**	8	5.6	5.5	3	9
reported litter size **Kelusa**	161	3.2	3	1	7
reported litter size **Antiga**	130	3.3	3	1	9

Appendix 2.5 Age structure of the study population (see graphs below).

Owner reported ages were often inconsistent. Therefore, population age structures were determined from the age distributions at the last time point, which contain the largest fraction of adults with known ages. Most dogs in their 42nd month of life or less had been observed as a pup or juvenile during the study period, so their true age was known. Additionally, several (Zenzele n= 9, Braamfischerville n= 4, Kelusa n= 9 and Antiga n=3) litters were born during the last time point.



Appendix 2.5 Age structures for the registered population were determined from the age distribution at the last time point, which consists of the largest fraction of adults with known ages. The x-axis shows the number of individuals in each age class and the y-axis shows the month of life. For example, dogs in age class 2-3 are in their second or third month of life.

Appendix 2.6 Confinement (see table below).

The method of confinement was variable. Dogs were confined, i.e. prevented from free-roaming, by tethers, pens, dog proof fences or a combination of these. “Frequently confined” dogs were reported at each interview for the entire period they were in the study population to have been confined during the previous seven days, although the frequency and duration of release during these seven days was variable. Confinement was also observed by the enumerators and the continuity of confinement between censuses discussed with the respondents. Very few dogs were never released.

Appendix 2.6 Confinement status of the registered populations.

	Zenzele	Braamfischerville	Kelusa	Antiga[b]
confined at some point while in the study population[a]	308 (30.1%)	551 (62.5%)	80 (11.3%)	80 (12.7%)
confined continuously or frequently while in the study population	<10%	~ 40%	<10%	<10%
never confined	548 (53.6%)	106 (12.0%)	568 (80.3%)	492 (78.2%)
missing information	166 (16.2%)	225 (25.5%)	59 (8.3%)	57 (9.1%)

[a] including dogs confined continuously or frequently while in the study population; [b] including 2011 data

Appendix 2.7 Sterilization status of the registered populations.

	Zenzele[a]	Braamfischerville[b]	Kelusa[c]	Antiga[d]
males sterilized	7 (0.7%)	50 (5.7%)	100 (14.1%)	169 (26.9%)
females sterilized	9 (0.9%)	50 (5.7%)	1 (0.1%)	1 (0.2%)
not sterilized	773 (75.6%)	593 (67.2%)	489 (69.2%)	359 (57.1%)
"don't know"	9 (0.9%)	19 (2.2%)	0	0
missing information	224 (21.9%)	169 (19.2%)	117 (16.5%)	100 (15.9%)

[a] most dogs were acquired sterilized from boss; [b] n=101 sterilized however the gender of one of these dogs was not known;
[c] males castrated "traditionally" and the one female was acquired sterilized from boss; [d] including 2011 data

Appendix 2.8 Size and density of the registered populations.

	study area km^2	median population size	population mean		population minimum			population maximum			linear regression		
			size	density km^{-2}	size	density km^{-2}	variation from mean	size	density km^{-2}	variation from mean	slope	SE	p-value
Zenzele	0.72	321	323.03	448.65	285	395.83	11.8%	362	502.78	12.1%	-1.524	0.232	<0.001
Braamfischerville	1.26	285	283.08	224.67	256	203.17	9.6%	294	233.33	3.8%	0.078	0.142	0.586
Kelusa	0.66	291	288.97	437.83	252	381.82	12.8%	312	472.73	8.0%	0.160	0.220	0.474
Antiga[a]	0.93	265	256.13	275.41	205	220.43	20.0%	286	307.53	11.7%	-1.364	0.283	<0.001
Kelod / Kaler	0.30	180.5	174.50	581.67	136	453.33	22.1%	195	650.00	11.7%	_	_	_
Ketug	0.63	84	81.50	129.37	66	104.76	19.0%	94	149.21	15.3%	_	_	_

Linear regression models were fitted to assess the overall trend in the population size with time; the slope, error and p-value are shown
[a] including the sub-villages (banjars) of Kelod / Kaler and Ketug; linear regression was applied at the village level only

Appendix 2.9 Age class at registration (excluding the starting cohorts).

	pups	juveniles	adults	age not reported
Zenzele	549 (81.6%)	42 (6.2%)	75 (11.1%)	7 (1.0%)
Braamfischerville	433 (71.1%)	73 (12.0%)	93 (15.3%)	10 (1.6%)
Kelusa	405 (89.0%)	28 (6.2%)	20 (4.4%)	2 (0.4%)
Antiga[a]	323 (84.1%)	30 (7.8%)	30 (7.8%)	1 (0.3%)

[a] 2011 data included

Appendix 2.10 Number of dogs in the registered populations gained per month (excluding the starting cohorts).

	mean	median	minimum	maximum
Zenzele	18.69	18	6	42
Braamfischerville	16.46	15	7	41
Kelusa	13.79	11	6	31
Antiga	10.32	9	2	28

Appendix 2.11 Number of dogs lost from the registered populations per month (excluding the starting cohorts).

	mean	median	minimum	maximum
Zenzele	20.31	19	7	34
Braamfischerville	16.14	16	3	38
Kelusa	13.55	13	3	25
Antiga	11.35	10	2	22

Appendix 2.12 and 2.13 Reproductively capable bitches (see tables below).

Bitches were observed to be reproductively capable (i.e. in oestrus, which resulted in pregnancy) during their eighth and seventh month of life in the Johannesburg and Bali sites respectively. Reproductive efficiency was assessed in bitches across all four sites, by examining the monthly distribution of dogs pregnant as a proportion of reproductively capable females. The observed and estimated (i.e. which assumed a proportion of dogs lost the following month were early pregnant) distributions were similar, although pregnancy rates were higher in Bali. There was no overall increase or decrease in the proportion of dogs pregnant and the number of litters per month, and there was no clear evidence of seasonal variation. Interpretation of the time series analysis is constrained by small numbers of females pregnant or whelping per month, although plots for Kelusa and Antiga and autocorrelation and partial autocorrelation for Antiga indicates periodicity with an interval of two months. This may be a consequence of the enumerators tending to record bitches whelping the month of the survey rather than the correct month within the usual 6 week inter-survey period for households with female dogs. This noise was not evident in the plots for population size for Bali (Figure 2.1) probably because, compared to all sources of registered dogs, only a small proportion originated from within the study area (Table 2.1).

In Zenzele 48.7%, Braamfischerville 63.6%, Kelusa 41.6% and Antiga 33% dogs categorised as reproductively capable did not reproduce during the study period, however these dogs encompass a range of ages, observational periods, reproductive histories, husbandry practices and health status. Consequently, differentiating the factors influencing fecundity, such as nutritional status, pathogens (Noakes, Parkinson & England 2009) and husbandry is not straight forward. In contrast to a previous study (Ortega-Pacheco *et al.* 2007b), on average pregnant dogs were more likely to be in a higher body condition category than non-pregnant dogs however this relationship does not vary across body condition class (Appendices 2.26-2.30). Despite careful enumerator training in body condition scoring, this variation may be the result of incorrect body condition scores allocated to pregnant dogs with enlarged abdomens. Furthermore, this model does not account for longitudinal variations in body condition prior to pregnancy necessary to determine the effect on body condition on the probability of pregnancy.

Appendix 2.12 Proportion of reproductively capable females pregnant per month in the registered populations.

	Zenzele		Braamfischerville		Kelusa		Antiga	
	observed proportion pregnant (number)	estimated proportion pregnant	observed proportion pregnant (number)	estimated proportion pregnant	observed proportion pregnant (number)	estimated proportion pregnant	observed proportion pregnant (number)	estimated proportion pregnant
mean	0.090 (8.49)	0.097	0.046 (3.34)	0.049	0.117 (4.94)	0.123	0.111 (3.91)	0.114
median	0.087 (8)	0.091	0.045 (3)	0.049	0.107 (5)	0.120	0.083 (3)	0.091
minimum	0.011 (3)	0.028	0 (0)	0	0 (0)	0	0.024 (1)	0.026
maximum	0.227 (20)	0.231	0.107 (8)	0.108	0.306 (12)	0.316	0.250 (9)	0.250
slope	2.89E-05	3.49E-05	1.04E-05	1.29E-05	3.00E-04	6.00E-04	-6.13E-05	-5.66E-05
SE	2.23E-05	2.11E-05	1.31E-05	1.29E-05	1.40E-03	1.40E-03	3.95E-05	3.97E-05
p-value	0.202	0.107	0.433	0.327	0.828	0.702	0.131	0.164

The estimated proportion pregnant includes the observed proportion pregnant plus an estimate of the proportion of females lost from the study population the following month that were early pregnant (i.e. lost before pregnancy could be confirmed visually)
Linear regression models were fitted to assess the overall trend in the proportion pregnant with time; the slope, error and p-value are shown

Appendix 2.13 Number of litters per month in the registered populations.

	Zenzele	Braamfischerville	Kelusa	Antiga
mean	8.38	3.42	5.35	4.18
median	8	3	5	3
minimum	3	0	0	1
maximum	20	8	12	9
slope	0.062	-0.004	0.053	-0.051
SE	0.061	0.031	0.056	0.042
p-value	0.320	0.910	0.355	0.241

Linear regression models were fitted to assess the overall trend in the number of litters per month; the slope, error and p-value are shown

Appendix 2.14 – 2.16 Mortality of registered dogs (see tables below).

With regards mortality, the category of "other" combines disease (including injury) -induced mortality and dogs found dead or where the cause of mortality was not known; based on Appendix 2.16 the majority of the dogs in the latter categories probably died from disease or injury.

There was no overall increase or decrease in the proportion of dogs dying per month, except for Kelusa where total mortality and combined reported and assumed (i.e. "other") disease-induced mortality increased ($p<0.001$). There was a marginal increase in reported (i.e. "specific") disease-induced mortality ($p=0.08$). There was no specific cause for the increase in total mortality in Kelusa; the number of dogs hit-by-cars (n=70) more than doubled in 2009 and 2010 (mean 2.5 deaths per month), the number of dogs killed by people (n=18) progressively increased from 0 per month in 2008 to 1 per month in 2010, and the number of dogs with missing information (n=36) doubled in 2010 to 1.7 per month. There is no clear evidence of periodicity, including seasonal variation, for overall mortality, specific disease-induced mortality and "other" mortality. Interpretation of the time series is constrained by the small numbers of dogs dying per month.

Although the clinical signs at the time of death were reported, in most cases the aetiology could not be determined. Rather, the reported clinical signs primarily served to verify that mortality was from illness rather than some other cause (Appendix 2.16). Mortality due to starvation (12% pups) has only been reported once (Pal 2001). In this study, body condition at the time of death was not consistently reported given the practical difficulties of verification by the enumerators. On average animals suffering from clinical conditions likely to cause weight loss were indeed thinner than animals not suffering from these conditions (Appendices 2.25-2.30), and this relationship did not vary with body condition class. Thus, weight loss would be expected in dogs suffering from these conditions at the time of death. Loss of body condition in sick dogs may be a consequence of nausea and inappetence rather than increased metabolic requirements. Concomitant neglect may also occur, particularly for dogs with generalised dermatitis, often with malodourous skin. Almost all of the dogs with generalised dermatitis were in Bali, and were the majority of those dogs with observed clinical signs (Appendices 2.26, 2.29-2.30). Owners only occasionally reported mortality from starvation (Appendix 2.16). More dogs were observed to be severely underweight (body condition score ≤ 2) than reported to have died from neglect.

Appendix 2.14 Mortality per month for the registered populations (includes all age classes).

	Zenzele						Braamfischerville						Kelusa						Antiga					
	total		disease		other		total		disease		other		total		disease		other		total		disease		other	
	number	prop.	number	prop.	number	prop.	number	prop.	number	prop.	number	prop.	number	prop.	number	prop.	number	prop.	number	prop.	number	prop.	number	prop.
mean	14.25	0.044	9.19	0.028	12.64	0.039	9.59	0.034	6.11	0.022	8.76	0.031	9.76	0.034	5.58	0.019	6.79	0.023	7.73	0.030	4.78	0.018	5.59	0.022
median	12	0.041	8	0.026	12	0.040	9	0.033	5	0.019	8	0.029	10	0.034	5	0.017	6	0.021	8	0.030	5	0.019	6	0.022
minimum	5	0.017	3	0.009	3	0.010	3	0.010	1	0.003	2	0.007	2	0.008	2	0.008	2	0.012	2	0.008	1	0.004	0	0
maximum	26	0.081	20	0.057	23	0.072	23	0.079	13	0.045	21	0.072	19	0.061	11	0.038	14	0.047	14	0.060	11	0.039	12	0.043
slope	1.90E-03	1.05E-05	1.20E-03	6.70E-06	1.70E-03	9.36E-06	-2.00E-03	-7.03E-06	1.00E-03	3.30E-06	-7.00E-04	-2.63E-06	8.19E-03	2.79E-05	2.60E-03	8.32E-06	4.50E-03	1.42E-05	6.00E-04	6.35E-06	-9.60E-04	-1.51E-06	-2.00E-04	1.79E-06
SE	3.20E-03	9.66E-06	2.30E-03	6.97E-06	2.80E-03	8.34E-06	2.10E-03	7.40E-06	1.50E-03	5.40E-06	2.10E-03	7.26E-06	2.01E-03	6.77E-06	1.40E-03	4.63E-06	1.50E-03	5.12E-06	1.70E-03	5.92E-06	1.20E-03	4.27E-06	1.40E-03	5.07E-06
p-value	0.570	0.281	0.615	0.343	0.557	0.269	0.344	0.348	0.536	0.545	0.735	0.720	<0.001	<0.001	0.074	0.082	0.005	<0.001	0.701	0.290	0.424	0.726	0.875	0.727

Linear regression models were fitted to assess the overall trend in the number and proportion dying with time; the slope, error and p-value are shown

Note: “other” combines disease (including injury)-induced mortality and dogs found dead or where the cause of mortality was not known on the basis that the majority of the dogs in the latter categories probably died from disease or injury (Appendix 2.16).

Appendix 2.15 Mortality per month of dogs ≤ 12 months (i.e. ~52 weeks) of age for the registered populations.

	Zenzele						Braamfischerville						Kelusa						Antiga					
	total		disease		other		total		disease		other		total		disease		other		total		disease		other	
	number	prop.	number	prop.	number	prop.	number	prop.	number	prop.	number	prop.	number	prop.	number	prop.	number	prop.	number	prop.	number	prop.	number	prop.
mean	6.58	0.021	4.19	0.013	6.03	0.019	4.49	0.016	3.03	0.011	4.22	0.015	4.58	0.016	2.67	0.009	3.24	0.011	3.70	0.014	2.46	0.001	2.97	0.012
median	6	0.019	4	0.013	5.5	0.018	4	0.014	3	0.010	4	0.014	3	0.011	2	0.007	3	0.010	3	0.014	3	0.010	3	0.011
minimum	2	0.006	1	0.003	2	0.006	1	0.003	0	0	1	0.003	1	0.004	0	0	0	0	1	0.004	0	0	0	0
maximum	12	0.038	10	0.032	10	0.034	14	0.048	7	0.025	13	0.045	10	0.036	8	0.027	8	0.027	10	0.035	8	0.028	9	0.032
slope	8.25E-02	2.40E-04	4.85E-02	1.50E-04	7.63E-02	2.17E-04	-1.97E-02	-6.57E-05	2.51E-02	9.58E-05	-2.49E-02	-8.63E-05	1.61E-01	5.55E-04	8.56E-02	2.90E-04	1.09E-01	3.74E-04	1.19E-02	9.25E-05	1.07E-02	9.37E-05	1.61E-02	1.25E-04
SE	5.38E-02	1.80E-04	3.66E-02	1.20E-04	4.64E-02	1.55E-04	4.46E-02	1.57E-04	3.21E-02	1.15E-04	4.31E-02	1.50E-04	3.80E-02	1.32E-04	3.00E-01	3.86E-04	3.23E-02	1.10E-04	3.43E-02	1.19E-04	2.69E-02	9.93E-05	2.90E-02	1.07E-04
p-value	0.134	0.192[a]	0.193	0.205[a]	0.109	0.172[a]	0.662	0.678	0.439	0.409	0.567	0.575	0.00018	0.0002	0.00765	0.0079	0.00199	0.00184	0.731	0.442	0.694	0.352	0.581	0.251

[a] including March & April 2008 p-values for total = 0.057, disease = 0.048, other = 0.041

Linear regression models were fitted to assess the overall trend in the number and proportion dying with time; the slope, error and p-value are shown

Note: “other” combines disease (including injury)-induced mortality and dogs found dead or where the cause of mortality was not known on the basis that the majority of the dogs in the latter categories probably died from disease or injury (Appendix 2.16).

Appendix 2.16 Causes of mortality of the registered dogs.

	Zenzele	Braamfischerville	Kelusa	Antiga
sick[a]	328 (63.9%)	218 (61.4%)	176 (54.7%)	157 (59.5%)
hit-by-car	37 (7.2%)	21 (5.9%)	70 (21.7%)	37 (14.0%)
killed by owner	4 (0.8%)	-	6 (1.9%)	5 (1.9%)
killed by someone	5 (1.0%)	3 (0.8%)	12 (3.7%)	18 (6.8%)
severe weather	-	2 (0.6%)	-	-
neglect / starvation	3 (0.6%)	-	1 (0.3%)	4 (1.5%) [b]
ceremonial sacrifice	NA	NA	6 (1.9%)	3 (1.1%)
eaten by the owner	NA	NA	-	4 (1.5%)
found dead	23 (4.5%)	19 (5.3%)	5 (1.4%)	2 (0.8%)
dumped	-	-	3 (0.8%)	5 (1.9%)
other	15(2.9%)	14 (3.9%)	7 (2.2%)	5 (1.9%)
don't know	21 (4.1%)	17 (4.8%)	21 (6.5%)	2 (0.8%)
missing entries	77 (15.0%)	61 (17.2%)	15 (4.7%)	22 (8.3%)
	513	355	322	264

[a] includes infections and injuries; [b] includes 3 pups that did not nurse properly

Appendix 2.17 – 2.19 Reasons for sourcing of dogs outside of the research sites (see tables below).

Across all communities, the vast majority of owners reported that getting a dog was a conscious decision. Many of the owners sought dogs from outside the community that they lived in; of those owners who planned to get a dog and sourced it from outside the research site, <50% (Zenzele 48%, Braamfischerville 38%, Kelusa 32%, Antiga 30%) tried to find a dog in the community that they lived in. Different cultural perspectives meant that determining why a dog was not sourced from inside the research site was difficult, hence the large amount of missing information. Owners who did not plan to get their dogs generally acquired them opportunistically, but the majority of these dogs were not unwanted; rather, at the time of acquisition owners deliberately decided that they wanted to keep their dogs for specific purposes, e.g. security.

Appendix 2.17 Reasons for sourcing a dog from outside the Johannesburg sites.

[a] Question: why did you not the dog from inside the research site?	**Zenzele**	**Braamfischerville**
	%	%
chance acquisition	16.8	21.1
gift	16.3	16
relocated with owner	12.4	3.4
rescued	3.5	1.7
stray / found	0.5	0.8
concerned the dog will return to the original owner	1.5	0.0
a dog was not available	6.9	5.9
a pup was not available	9.4	14.8
an adult was not available	1.0	0.0
the right breed was not available	11.9	21.5
from a breeder	0.0	0.4
the available breed was preferred	1.5	1.7
a healthy dog was not available	6.4	8.0
dogs in the research site are unhealthy	2.5	1.3
dogs in the research site are unclean	1.5	0.0
a dog with the right temperament was not available	5.0	7.6
a male was not available	1.5	3.8
a female was not available	1.0	0.0
dog sitting	1.5	0.8
convenient to get from relative	3.5	11.8
convenient to get from friend	4.0	12.2
from boss or relative's / work / colleague	1.2	7.6
from stranger	0.0	0.4
did not want to ask friends / family / neighbours in the research site	0.5	4.2
did not know who to ask in the research site	0.0	8.9
brought home	1.0	0.4
free dog not available in BF / dog was free	0.0	1.7
no time to look in the research site / dog readily available	0.5	0.0
from a second house	0.0	0.8
other	0.5	2.1
don't know	3.0	0.0

[a] either could not find a dog or assumed that a dog could not be found

Information was available for 73.5% (202/275) of the dogs in Zenzele and 68.7% (237/345) of the dogs in Braamfischerville. Most of the missing information is prior to November 2009 when the relevant questions were changed to improve interpretation.

Appendix 2.18 Reasons for sourcing a dog from outside the Bali sites.

[a] Question: why did you not get the dog from inside the research site?	**Kelusa**	**Antiga**
	%	%
chance acquisition	39.3	49.0
gift	3.6	6.3
rescued	0.0	1.0
stray / found	2.7	0.0
a dog was not available	7.1	2.1
a pup was not available	5.4	2.1
the right breed was not available	11.6	12.5
the available breed was preferred	0.0	5.2
a healthy dog was not available	8.9	9.4
the available dog was healthy	0	1.0
a dog with the right temperament was not available	7.1	1.0
the available dog was the right temperament	0.0	2.1
a male was not available	3.6	1.0
a male dog was available	0.0	1.0
convenient to get from a relative	22.3	30.2
convenient to get from a friend	25.0	15.6
from boss or relative's / work / colleague	4.5	6.3
did not want to ask friends / family / neighbours in the research site	1.8	2.1
brought home	1.8	0
for ceremony	0.0	2.1
no time to look in the research site	0.0	5.2
from a second house	2.7	4.2

[a] either could not find a dog or assumed that a dog could not be found

Information was available for 76.2% (112/147) of the dogs in Kelusa and 69.1% (96/139) of the dogs in Antiga. Most of the missing information is prior to November 2009 when the relevant questions were changed to improve interpretation. These data exclude 7 dogs in Kelusa and 1 dog in Antiga born in the dry field and categorized as from outside the research site in Table 2.1.

Appendix 2.19 Assessment of whether the acquisition of the dog was planned and if the owner looked in the research site for a dog.

	Question: did you plan to get a dog?			Question: did you look for a dog in the research site?		
	yes	no	missing data	yes	no	missing data
Zenzele	83	12	180	38	39	198
Braamfischerville	158	16	171	60	96	189
Kelusa	47	29	71	15	56	76
Antiga	33	31	75	10	54	75

Most of the missing information is prior to November 2009 when these questions were added to the survey to improve interpretation of the question: Why did you not get the dog from inside the research site? (see Appendices 2.17 and 2.18)

Appendix 2.20 Registered dogs acquired from outside the study areas per month.

	Zenzele		Braamfischerville		Kelusa		Antiga	
	number	prop.	number	prop.	number	prop.	number	prop.
mean	7.57	0.023	9.63	0.034	5.09	0.018	4.67	0.018
median	7	0.022	8	0.029	5	0.017	3	0.012
minimum	2	0.006	3	0.010	0	0	0	0
maximum	21	0.060	21	0.074	11	0.040	13	0.049
slope	-6.90E-02	-1.13E-04	-1.55E-02	-7.06E-05	9.43E-02	3.25E-04	-0.01671	-6.62E-05
SE	6.32E-02	1.92E-04	6.45E-02	2.29E-04	4.70E-02	1.70E-04	0.06781	2.49E-04
p-value	0.282	0.561	0.811	0.760	0.054	0.064	0.807	0.792

Linear regression models were fitted to assess the overall trend in the number and proportion of registered dogs from outside the study area with time; the slope, error and p-value are shown

Appendix 2.21(i) Outcome of pups (see table below).

The number of pups born in households in the Bali study areas may be substantially higher than shown given that female neonates were often dumped and, thus, litter size was probably deliberately under-reported. Where the complete litter size was not known by an owner, litter size was rounded up based on the average number of foetuses observed in gravid uteri during routine surgery (Appendix 2.4). The complete litter size was not known for 9% of the litters born in Zenzele during the study period, 7% in Braamfischerville 9% in Kelusa and 6% in Antiga.

Appendix 2.21(ii) Unknown sources and outcomes of dogs (see table below and Tables 2.1-2.2).

Tables 2.1 and 2.2 (and Appendix 2.21) show the contribution of unknown sources and outcomes of dogs to the dynamics of the populations. Based on the distribution of known outcomes, the majority of dogs with unknown outcomes probably died given that they were not observed by the enumerators. This sub-group should not be ignored in population models or control policies. In addition, a proportion of the registered dogs from the study area but where the household of origin was not identified[a] were probably from the pool of locally born pups given away in the study area or research site and where the recipient household was not identified[b]. The residuals from the second pool of dogs[b] may have died or subsequently moved out of the study area given that they were not observed by the enumerators (Zenzele 98[a]: 138[b], Braamfischerville 4: 62, Kelusa 20: 55, Antiga 6: 88).

Appendix 2.21 Outcomes of the pups born in the study areas.

	Zenzele	Braamfischerville	Kelusa	Antiga
kept where born (registered)	128 (7.2%)	60 (7.8%)	128 (20.9%)	87 (18.1%)
given away in study area address known (registered)	112 (6.3%)	33 (4.3%)	24 (3.9%)	18 (3.7%)
total number pups born in study area registered	240 (13.5%)	93 (12.1%)	152 (24.8%)	105 (21.8%)
given away in study area address not known / pup not found	132 (7.4%)	8 (1.0%)	1 (0.2%)	0
given away in research site but area not known	NA	54 (7.0%)	54 (8.8%)	88 (18.3%)
given away in non-study area of the research site	NA	3 (0.4%)	8 (1.3%)	23 (4.8%)
given away but location not known	6 (0.3%)	0	0	0
given away out outside research site	103 (5.8%)	65 (8.5%)	30 (4.9%)	18 (3.8%)
died	1040 (58.6%)	438 (57.0%)	272 (44.4%)	179 (37.2%)
disappeared	25 (1.4%)	7 (0.9%)	6 (1.0%)	10 (2.1%)
stolen	27 (1.5%)	31 (4.0%)	0	0
relocated outside the research site with owner	13 (0.7%)	0	0	2 (0.4%)
relocated to non-study area of the research site with owner	NA	7 (0.9%)	0	0
unaccounted for	190 (10.7%)	63 (8.2%)	90 (14.7%)	56 (11.6%)
total number pups born in study area	1776	769	613	481

Includes pups born 1 or 2 months prior to the start of the study period that were present at the start of the study period

Appendix 2.22 Average time to loss or censoring of the starting cohorts.

	factor	level	average time (months)
Zenzele	age	adult	19.8
		juvenile	16.7
		pup	13.0
	gender	male	18.6
		female	19.0
Braamfischerville	age	adult	20.1
		juvenile	13.2
		pup	17.2
	gender	male	19.4
		female	18.0
Kelusa	age	adult	23.3
		juvenile	16.9
		pup	16.8
	gender	male	22.8
		female	18.4
Antiga	age	adult	26.7
		juvenile	18.4
		pup	16.2
	gender	male	26.2
		female	21.0

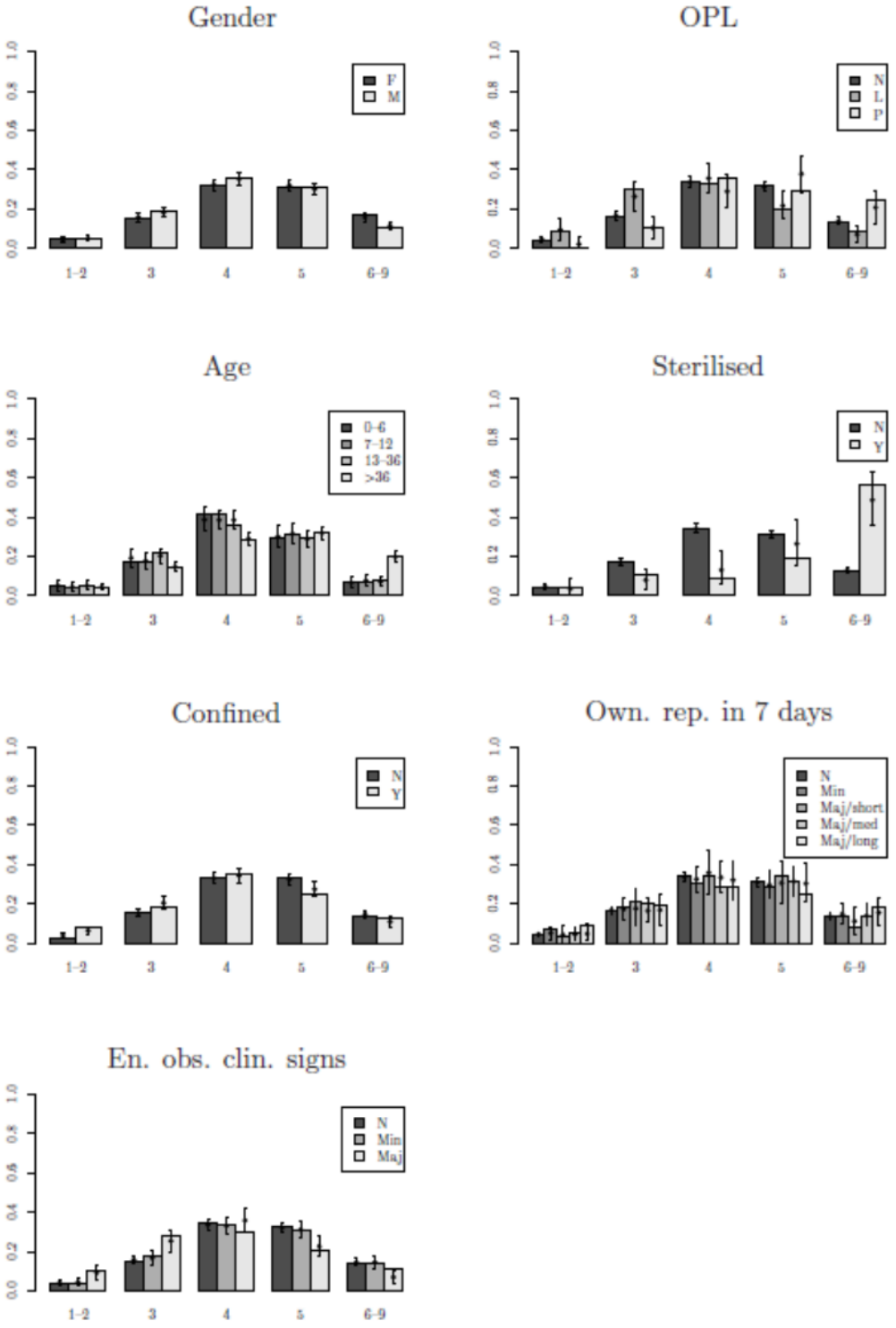
Appendix 2.23 Risk of loss from the starting cohorts by age class at the start of the study and gender.

	observations	study period (months)	factors retained in the minimum adequate model	factor level	exp(coef)	se(coef)	2.5%CI	97.5%CI	p-value
Zenzele	368	36	age	adult	-	-	-	-	-
				juvenile	1.315	0.145	0.990	1.748	0.059
				pup	1.761	0.253	1.074	2.889	0.025
Braamfischerville	287	36	age	adult	-	-	-	-	-
				juvenile	1.862	0.168	1.339	2.589	<0.001
				pup	1.396	0.289	0.793	2.458	0.248
Kelusa	278	33	age + gender	adult	-	-	-	-	-
				juvenile	1.777	0.201	1.197	2.637	<0.001
				pup	1.831	0.235	1.156	2.901	<0.001
				female	-	-	-	-	-
				male	0.582	0.164	0.422	0.802	<0.001
Antiga	266	37	age	adult	-	-	-	-	-
				juvenile	2.203	0.218	1.437	3.376	<0.001
				pup	2.749	0.262	1.646	4.590	<0.001

All the cox proportional hazards models included duration in the study, age class at the start of the study and gender. The model values vary slightly from the averages shown in Appendix 2.22 because of how the model handled censoring (Crawley 2007)

Appendix 2.24 Outcomes of the dogs in the starting cohorts.

	Zenzele	Braamfischerville	Kelusa	Antiga
total died	204 (71.1%)	145 (65.9%)	127 (72.2%)	116 (72.5%)
died of disease	132 (64.7%)	83 (57.2%)	82 (64.6%)	72 (62.1%)
disappeared	19 (6.6%)	5 (2.3%)	21 (11.9%)	13 (8.1%)
stolen	8 (2.8%)	12 (5.5%)	0	0
given away in non-study area of the research site	NA	1 (0.5%)	0	0
given away outside research site	16 (5.6%)	21 (9.5%)	5 (2.8%)	10 (6.3%)
given to meat trader	NA	NA	11 (6.3%)	7 (4.4%)
relocated outside research site with owner	15 (5.2%)	15 (6.8%)	3 (1.7%)	0
dumped	0	0	0	1 (0.6%)
other	1 (0.3%)	0	0	1 (0.6%)
unaccounted for	19 (6.6%)	14 (6.4%)	9 (5.1%)	10 (6.3%)
given away in study area but not found by enumerators	5 (1.7%)	1 (0.5%)	0	0
given away in research site but area not known	NA	6 (2.7%)	0	2 (1.3%)
total lost	287	220	176	160



Appendix 2.25 Marginal positive predictive distributions for the explanatory variables, including observed pregnancy and lactation, against the observed maximum body condition scores for Zenzele. Bars represent the data, the points are the marginal predictive means and the error bars are the 95% prediction intervals. These distributions are representative of the four communities, including maximum and minimum body condition scores and arbitrary and observed pregnancy and lactation. OPL includes non-lactating females and males (N) and lactating (L) and pregnant (P) dogs. Age categories are in months (see Appendix 2.26).

Appendix 2.26 Variables included in the ordinal regression models. See Appendix 2.27 for application of the variable categories.

variable	categories
body condition score	1&2 (emaciated & very underweight), 3 (underweight), 4 (slightly underweight), 5 (ideal), 6-9 (slightly overweight - obese); maximum body condition = largest of the two condition scores; minimum body condition = smallest of the two condition scores
gender	female vs. male
lactating	non-lactating female and male vs. lactating female; arbitrary lactation period = 12 weeks lactation period; observed lactation period = lactation observed by the enumerators
pregnant	non-pregnant female and male vs. pregnant female; arbitrary pregnancy period = 63 days gestation; observed pregnancy period = pregnancy observed by the enumerators
sterilized	not sterilized vs. sterilized
confined	not confined at some point since the previous census vs. confined at some point during the previous census
age	1-6 months[a] vs. 7-12 months, 13-36 months, mature adult[b]
number of dogs per household	number of dogs per household
observed minor clinical signs	healthy vs. clinical conditions unlikely to cause weight loss observed by the enumerators
observed major clinical signs	healthy vs. clinical conditions likely to cause weight loss (and generalised dermatitis); includes dogs with a combination of minor and major clinical signs
owner reported minor clinical signs	healthy vs. only clinical conditions unlikely to cause weight loss for the previous 7 days and 3 months (i.e. since the last census)
owner reported major clinical signs	healthy vs. clinical conditions likely to cause weight loss (and generalised dermatitis) for the previous 7 days and 3 months (i.e. since the last census); includes dogs with a combination of minor and major clinical signs; short duration = one episode or one day duration, medium = 2-6 days duration, long = 7+ days duration

[a] dogs in age class 1-6 months are between birth [beginning of their 1st month of life] and ~26 weeks of age [end of their 6th month of life], dogs in age class 7-12 months are between ~27 weeks of age [beginning of their 7th month of life] and ~52 weeks of age [end of their 12th month of life], and dogs in age class 13-36 months are between ~53 weeks of age [beginning of their 13th month of life] and ~156 weeks of age [end of their 36th month of life];

[b] mostly dogs first observed as adults at the start of the study period, but also dogs first observed as adults after the start of study period, therefore the exact age of these dogs could not be determined by direct observation [Note: dogs in age classes 1-6, 7-12 and 13-36 months were observed as pups or juveniles during the study period, so their true age was known]

Appendix 2.27 Zenzele ordinal regression model averaged posterior means and standard deviations for the log cumulative odds ratios.

Zenzele	maximum body condition score					maximum body condition score					minimum body condition score					minimum body condition score				
	PO	NPO	excluded	mean	SD	PO	NPO	excluded	mean	SD	PO	NPO	excluded	mean	SD	PO	NPO	excluded	mean	SD
gender:male	0.69	0	0.31	-0.27	0.22	0.53	0	0.47	-0.19	0.21	0.71	0	0.29	-0.28	0.21	0.73	0	0.27	-0.28	0.21
lactating: arbitrary lactation period	1.00	0	0	-0.89	0.16	_	_	_	_	_	1.00	0	0	-0.94	0.16	_	_	_	_	_
lactating: observed lactation period	_	_	_	_	_	1.00	0	0	-1.10	0.18	_	_	_	_	_	1.00	0	0	-1.30	0.18
pregnant: arbitrary gestation period	1.00	0	0	0.23	0.18	_	_	_	_	_	1.00	0	0	0.45	0.18	_	_	_	_	_
pregnant: observed gestation	_	_	_	_	_	1.00	0.002	0	(0.46, 0.45, 0.45, 0.45)	(0.25, 0.22, 0.22, 0.22)	_	_	_	_	_	1.00	0	0	0.47	0.21
7-12 months	1.00	0	0	0.12	0.15	1.00	0	0	0.13	0.15	0.94	0.0005	0.06	0.30	0.17	1.00	0.0005	0	0.34	0.15
13-36 months	1.00	0	0	(-0.16, -0.17, -0.16, -0.16)	0.16	1.00	0	0	-0.18	0.16	0.94	0	0.06	0.065	0.15	1.00	0	0	0.09	0.15
mature adult	0	1.00	0	(-0.006, 0.27, 0.75, 1.50)	(0.23, 0.18, 0.17, 0.21)	0	1.00	0	(-0.003, 0.27, 0.75, 1.50)	(0.23, 0.18, 0.18, 0.21)	0.84	0.096	0.06	(0.62, 0.65, 0.70, 0.73)	(0.28, 0.24, 0.25, 0.31)	0.88	0.12	0	(0.68, 0.72, 0.78, 0.83)	(0.25, 0.18, 0.19, 0.27)
confined	0.99	0.005	0.002	(-0.53, -0.52, -0.52, -0.52)	(0.13, 0.12, 0.12, 0.13)	0.99	0.009	0	(-0.56, -0.55, -0.55, -0.55)	(0.13, 0.11, 0.11, 0.12)	1.00	0.0005	0.0015	-0.54	0.12	1.00	0.0015	0	-0.57	0.12
observed minor clinical signs	1.00	0	0	-0.14	0.11	1.00	0	0	-0.14	0.10	1.00	0	0	-0.15	0.11	1.00	0	0	-0.14	0.11
observed major clinical signs	1.00	0.001	0	-1.00	0.15	1.00	0.005	0	-1.00	(0.15, 0.15, 0.15, 0.16)	1.00	0.0005	0	-1.30	(0.14, 0.14, 0.15, 0.15)	1.00	0	0	-1.30	0.15

The first three columns (Proportional Odds [PO], Non-Proportional Odds [NPO] and excluded) show the posterior probabilities of association (PPA) for each variable, averaged across all the competing models. Only those variables with a PPA ≥0.5 are included (Jefferys 1961). The averaged posterior means and standard deviations for each variable are also shown. Where the means and SDs are the same across the levels (1-4) only a single result is shown.

In reference to the results for observed lactation and gestation and maximum and minimum body condition scores, males are on average ~1.2× more likely to have a lower body condition score (BCS) than females. Lactating females are, on average, ~ 2.2× more likely to have a lower BCS than equivalent males and non-pregnant females. Pregnant females are ~1.5× more likely to be have a higher BCS than non-pregnant females and males. For age, relative to the 1–6 month category, dogs aged 7–12 months are ~1.2× more likely to have a higher BCS; and, dogs aged 13–36 months are ~1-1.2× more likely to have a lower BCS. The mature adult age class has very strong evidence of an NPO structure. Mature adults are ~1–2.5× more likely to have a higher BCS, depending on the category level. For maximum BCS, mature dogs were clustered in the higher body condition categories; whereas, for the minimum BCS, these dogs were distributed across body condition categories but were, on average, fatter than dogs in the other age categories. A likely explanation is that this pattern reflects normal morphological variation - generally, as dogs become older their activity levels will decrease, resulting in a general increase in BCS. Dogs with major and minor clinical sings were ~2x and 1.1x more like to have a lower BCS than healthy dogs respectively.

Appendix 2.28 Braamfischerville ordinal regression model averaged posterior means and standard deviations for the log cumulative odds ratios; see Appendix 2.27 for interpretation of the results.

Braamfischerville	maximum body condition score					maximum body condition score					minimum body condition score					minimum body condition score				
	PO	NPO	excluded	mean	SD	PO	NPO	excluded	mean	SD	PO	NPO	excluded	mean	SD	PO	NPO	excluded	mean	SD
lactating: arbitrary lactation period	0.75	0	0.25	-0.69	0.47	_	_	_	_	_	0.94	0	0.058	-1.00	0.38	_	_	_	_	_
lactating: observed lactation period	_	_	_	_	_	0.98	0	0.019	-1.20	0.37	_	_	_	_	_	1.00	0	0.004	-1.50	0.35
pregnant: arbitrary gestation period	0.75	0	0.25	0.16	0.27	_	_	_	_	_	0.94	0.0005	0.058	(0.0095, 0.0095, 0.009, 0.0095)	0.27	_	_	_	_	_
pregnant: observed gestation	_	_	_	_	_	0.98	0.001	0.019	0.51	0.42	_	_	_	_	_	1.00	0.0005	0.004	0.24	(0.39, 0.39, 0.39, 0.40)
7-12 months	0.96	0	0.036	0.24	0.22	0.99	0	0.0065	0.26	0.21	_	_	_	_	_	_	_	_	_	_
13-36 months	0.96	0	0.036	-0.24	0.22	0.99	0	0.0065	-0.24	0.22	_	_	_	_	_	_	_	_	_	_
mature adult	0.96	0	0.036	0.64	0.28	0.99	0	0.0065	0.67	0.24	_	_	_	_	_	_	_	_	_	_
sterilised	0.68	0	0.32	0.42	0.35	0.70	0	0.30	0.44	0.35	0.52	0.0005	0.48	0.28	0.31	0.59	0.0005	0.41	0.33	0.33
observed minor clinical signs	1.00	0	0	-0.23	0.15	1.00	0	0	-0.24	0.15	1.00	0	0	-0.36	0.15	1.00	0	0	-0.38	0.15
observed major clinical signs	1.00	0	0	-1.40	0.21	1.00	0.0005	0	-1.50	0.21	1.00	0	0	-1.30	0.21	1.00	0	0	-1.30	0.22

The first three columns (PO, NPO and excluded) show the posterior probabilities of association (PPA) for each variable, averaged across all the competing models. Only those variables with a PPA ≥0.5 are included (Jefferys 1961). The averaged posterior means and standard deviations for each variable are also shown. Where the means and SDs are the same across the levels (1-4) only a single result is shown.

Appendix 2.29 Kelusa ordinal regression model averaged posterior means and standard deviations for the log cumulative odds ratios; see Appendix 2.27 for interpretation of the results.

Kelusa	maximum body condition score					maximum body condition score					minimum body condition score					minimum body condition score				
	PO	NPO	excluded	mean	SD	PO	NPO	excluded	mean	SD	PO	NPO	excluded	mean	SD	PO	NPO	excluded	mean	SD
lactating: arbitrary lactation period	1.00	0	0	-0.92	0.23	_	_	_	_	_	1.00	0.001	0.001	-0.87	(0.23, 0.22, 0.23, 0.24)[b]	_	_	_	_	_
lactating: observed lactation period	_	_	_	_	_	1.00	0	0	-1.20	0.23	_	_	_	_	_	1.00	0.001	0	-1.20	0.24
pregnant: arbitrary gestation period	1.00	0	0	0.36	0.24	_	_	_	_	_	1.00	0	0.001	0.40	0.24	_	_	_	_	_
pregnant: observed gestation	_	_	_	_	_	1.00	0	0	0.96	0.35	_	_	_	_	_	1.00	0	0	1.10	0.36
6-12 months of age	1.00	0	0	-0.83	0.17	1.00	0	0	-0.85	0.17	1.00	0.0005	0	-0.93	0.18	1.00	0	0	-0.94	0.17
13-36 months of age	1.00	0	0	-1.40	0.19	1.00	0	0	-1.40	0.19	1.00	0.002	0	-1.50	0.20	1.00	0	0	-1.50	0.19
mature adult	1.00	0	0	-0.46	0.21	1.00	0	0	-0.48	0.21	1.00	0	0	-0.51	0.22	1.00	0	0	-0.51	0.22
sterilised	1.00	0	0	1.70	0.23	1.00	0	0	1.70	0.22	1.00	0.001	0	1.70	(0.23, 0.22, 0.22, 0.22)[b]	1.00	0	0	1.70	0.23
confined	1.00	0	0.0015	1.20	0.27	1.00	0	0	1.20	0.26	1.00	0	0	1.40	0.27	1.00	0	0	1.40	0.27
observed minor clinical signs	1.00	0	0	-0.50	0.16	1.00	0	0	-0.51	0.16	1.00	0	0	-0.55	0.17	1.00	0	0	-0.56	0.16
observed major clinical signs	1.00	0	0	-0.88	0.13	1.00	0	0	-0.88	0.13	1.00	0	0	-1.10	0.13	1.00	0	0	-1.10	0.13

The first three columns (PO, NPO and excluded) show the posterior probabilities of association (PPA) for each variable, averaged across all the competing models. Only those variables with a PPA ≥0.5 are included (Jefferys 1961). The averaged posterior means and standard deviations for each variable are also shown. Where the means and SDs are the same across the levels (1-4) only a single result is shown.

Appendix 2.30 Antiga ordinal regression model averaged posterior means and standard deviations for the log cumulative odds ratios; see Appendix 2.27 for interpretation of the results.

Antiga	maximum body condition score					maximum body condition score					minimum body condition score					minimum body condition score				
	PO	NPO	excluded	mean	SD	PO	NPO	excluded	mean	SD	PO	NPO	excluded	mean	SD	PO	NPO	excluded	mean	SD
lactating: arbitrary lactation period	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
lactating: observed lactation period	_	_	_	_	_	0.85	0.001	0.15	-0.38	(0.29, 0.28, 0.29, 0.29)[b]	_	_	_	_	_	0.92	0.001	0.076	-0.41	0.28
pregnant: arbitrary gestation period	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
pregnant: observed gestation	_	_	_	_	_	0.85	0	0.15	0.83	0.49	_	_	_	_	_	0.92	0.0005	0.076	0.97	0.45
number of dogs per household	1.00	0	0.025	-0.31	0.07	0.99	0	0.01	-0.29	0.077	1.00	0	0	-0.35	0.072	1.00	0	0	-0.33	0.069
6-12 months of age	1.00	0	0	-0.67	0.23	1.00	0	0	-0.70	0.24	1.00	0	0	-0.72	0.23	1.00	0	0	-0.75	0.24
13-36 months of age	1.00	0	0	-1.50	0.24	1.00	0	0	-1.50	0.25	1.00	0	0	-1.40	0.25	1.00	0	0	-1.40	0.26
mature adult	1.00	0	0	-0.36	0.26	1.00	0	0	-0.37	0.27	1.00	0	0	-0.34	0.26	1.00	0	0	-0.32	0.27
sterilised	0.97	0	0.028	0.80	0.26	0.97	0	0.031	0.81	0.27	0.96	0	0.041	0.76	0.27	0.96	0	0.045	0.76	0.27
confined	0.95	0.04	0.007	1.10	(0.34, 0.36, 0.31, 0.33)[b]	0.96	0.035	0.004	1.10	(0.33, 0.33, 0.30, 0.31)[b]	0.96	0.001	0.041	0.94	0.34	0.95	0.0005	0.049	0.91	0.35
observed minor clinical signs	1.00	0	0	-0.25	0.19	1.00	0.001	0	-0.25	0.19	1.00	0	0	-0.33	0.19	1.00	0.0005	0	-0.32	0.19
observed major clinical signs	1.00	0	0	-0.90	0.13	1.00	0	0	-0.88	0.13	1.00	0	0	-0.86	0.13	1.00	0	0	-0.85	0.14

The first three columns (PO, NPO and excluded) show the posterior probabilities of association (PPA) for each variable, averaged across all the competing models. Only those variables with a PPA ≥0.5 are included (Jefferys 1961). The averaged posterior means and standard deviations for each variable are also shown. Where the means and SDs are the same across the levels (1-4) only a single result is shown.

Appendix 3

Appendix 3.1 Study methodology.

order	activity	level	process
1	introduction	banjar	▪ introduce facilitators ▪ state purpose of the session - to understand their perspective ▪ reiterate no right or wrong answers
2	semi-structured discussion - general	banjar	▪ feedback on the following questions: ▫ who has a dog? or, do you have a dog? ▫ how do you keep your dogs? (confined or unconfined) ▫ how do you identify an owned and unowned dog? ▫ what do you know about rabies? or, do you have any questions about rabies?
3	visualisation tool - body mapping	groups	▪ each group randomly allocated one of the following: ▫ healthy male dog, healthy female dog, unhealthy male dogs, unhealthy female dog ▪ each group asked to "Draw what you think is a healthy / unhealthy male / female dog" ▪ affected parts of the body are marked, or characteristics listed
		banjar	▪ each group shares what they have drawn with the rest of the banjar ▪ after each group, the banjar is asked if anything is missing from the drawings ▪ finally, while referring to the healthy dog picture, ask if the dog is still healthy if it has: i) a runny nose, ii) watering eyes, iii) ribs showing, iv) limps, v) ticks, vi) bad skin
4	semi-structured discussion - food sources	banjar	▪ ask what are the various things which affect a dog's health ▪ as soon as "food" is suggested, conversation is directed to sources ▪ four volunteers asked to draw each food source on a separate piece of paper as they come up in conversation
5	ranking - food sources	banjar	▪ first in reference to the healthy dog (picture hanging on a flipchart board) identify which food sources are relevant and irrelevant; and then rank the relevant food sources in order of importance (food source pictures semi-permanently tacked onto the picture of the healthy dog so they can be re-arranged as necessary) ▪ repeat the process for unhealthy, owned and unowned dogs
6	conclusion	banjar	▪ thank the banjar for their participation ▪ explain the (research) application of the information they provided ▪ ask if the banjar has any questions for the facilitators

Appendix 3.2 Approximate number of participants.

village	banjar	total number of participants	men	women	children
Antiga	**Kaler** (mixed)	110	95[a]	~15	0
	Ketug (mixed)	90	67	8[b]	~15 (<14y)
	Kelod (mixed)	84	32	32	20
Kelusa	**Kelikikawan** (mixed)	60	50	~ 10[c]	–
	Roban (male)	35	33	2	0
	Roban (female)	54	0	50	4
	Ayah (male)	41	41	0	0
	Ayah (female)	44	0	41	3
	Peliatan (male)	12	12	0	0
	Peliatan (female)	74	0	54[d]	4
	Triwangsa (male)	16	16	0	0
	Yehtengah (male)	92	92	0	0
	Yehtengah (female)	45	0	45	0

[a] includes 15 male youths; [b] includes 2-3 female youths; [c] including children; [d] includes ~8 male and 8 female young teenagers

Note: A small number of participants came and went during each session. Most children were ≤10 years of age and did not participate in the PRA exercises.

Appendix 3.3 Health status in relation to body condition for Antiga.

	Kaler (mixed)	**Ketug** (mixed)	**Kelod** (mixed)
group-level description of:			
healthy male dog	fat	fat	fat
healthy female dog	fat	strong, eats well	not done[a]
unhealthy male dog	skinny	not listed	skinny, weak
unhealthy female dog	missing entry	ribs showing	not done[a]
banjar-level response to the question:			
Is a dog still healthy if it looks like this (the healthy dog picture) but has ribs showing ? [b]	healthy	unhealthy	unhealthy

[a] low attendance due to Hindu ceremonies therefore the banjar was divided into only two groups; [b] see Table 3.1 for full details of this question

Appendix 3.4 Health status in relation to body condition for Kelusa.

	Kelikikawan (mixed)	**Roban** (male)	**Roban** (female)	**Ayah** (male)	**Ayah** (female)
group-level description of:					
healthy male dog	fat	fat	fat	not listed	fat
healthy female dog	fat	fat	fat, strong	not listed	fat, good appetite
unhealthy male dog	skinny	skinny	not listed	skinny	not listed
unhealthy female dog	not listed	not listed	skinny, no appetite	skinny	skinny
banjar-level response to the question:					
Is a dog still healthy if it looks like this (the healthy dog picture) but has ribs showing ? [b]	unhealthy	unhealthy [a]	unhealthy	unhealthy	unhealthy

	Peliatan (male)	**Peliatan** (female)	**Triwangsa** (male)	**Yehtengah** (male)	**Yehtengah** (female)
group-level description of:					
healthy male dog	fat	not listed	fat, good appetite	fat	not listed
healthy female dog	not listed	not listed	good appetite	fat	not listed
unhealthy male dog	not listed	not listed	not listed	not listed	not listed
unhealthy female dog	not listed	no appetite	not listed	skinny	skinny
banjar-level response to the question:					
Is a dog still healthy if it looks like this (the healthy dog picture) but has ribs showing ? [b]	unhealthy	unhealthy	unhealthy	unhealthy	unhealthy

[a] the majority of the banjar said unhealthy, however two banjar members disagreed and said healthy; [b] refer Appendix 3.1 for full details of this question

Appendix 3.5 Rank of food sources of healthy dogs in order of importance (1 = most important).

	Antiga			Kelusa									
	Kaler (mixed)	Ketug (mixed)	Kelod (mixed)	Kelikikawan (mixed)	Roban (male)	Roban (female)	Ayah (male)	Ayah (female)	Peliatan (male)	Peliatan (female)	Triwangsa[c] (male)	Yehtengah (male)	Yehtengah (female)
food prepared by owner for the dog	1	1	2	2	1	1	2	2	2	2	2	1	1
leftovers from the owner	4		1	2[b]	2	3	3	3	3	3	3	3	3
food purchased from pet shop by owner				1		2	1	1	1	1	1	2	2
leftovers from the neighbour	3		4	4					5				
stealing food from the neighbour					3								
school children					9								
offerings	2	2[a]	6	5	4			4	4				
rubbish	5												
spoiled food in the ditch	8												
school rubbish			5				4					5	
waste from the local stalls			3		8		5	6			4	4	
pig food from the sty	6				6								4
stealing food from the local stalls				6									
stealing poultry				3	5		6	5					
dead animals	7				7								
fish from the river				7									
cassava in dry fields			7										

[a] fresh offerings; [b] equal with food prepared by owner; [c] no women's community group in Banjar Triwangsa

Appendix 3.6 Rank of food sources of owned dogs in order of importance (1 = most important).

	Antiga			Kelusa									
	Kaler (mixed)	Ketug (mixed)	Kelod (mixed)	Kelikikawan (mixed)	Roban (male)	Roban (female)	Ayah (male)	Ayah (female)	Peliatan (male)	Peliatan (female)	Triwangsa (male)	Yehtengah (male)	Yehtengah (female)
food prepared by owner for the dog	4	1	4	2		1	1	2	1	2	1	1	1
leftovers from the owner	1		1	3	1	3	3	3	5	3	3	2	4
food purchased from pet shop by owner				1		2	2	1	2	1	2	4	2
leftovers from the neighbour			6	7					7				
stealing food from the neighbour					3								
school children					4								
offerings			3	6			5	4	4				
rubbish				10									
school rubbish			2				4		8				
waste from the local stalls			5		2			6	3			3	3
pig food from the sty	2	2		9	6								
stealing food from the local stalls				4									
stealing poultry				8				5				5	
dead animals	3	3			5				9				
fish from the river				5									
vermin									6				
faeces from livestock and children													5

Appendix 3.7 Rank of food sources of unhealthy dogs in order of importance (1 = most important).

	Antiga			Kelusa									
	Kaler (mixed)	Ketug (mixed)	Kelod (mixed)	Kelikikawan (mixed)	Roban (male)	Roban (female)	Ayah (male)	Ayah (female)	Peliatan (male)	Peliatan (female)	Triwangsa (male)	Yehtengah (male)	Yehtengah (female)
leftovers from the owner				6	6								
leftovers from the neighbour	4[a]												
stealing food from the neighbour					5								
offerings		6				4	4	4			5		
rubbish	3		2	4	1	1	3	3		3	3	2	3
spoiled food in the ditch	1						2						2
school rubbish		2				5			4				
waste from the local stalls		4				2					7		
waste food from someone other than the owner or neighbour		7											
pig food from the sty		3	3		3	3		5	2		6	4	
stealing poultry				5									
dead animals	2	5	1	2	4			1	1	2	1	3	1
fish from the river		1											
vermin				1					3				
faeces		1[c]		3	2								
livestock faeces							1	2		1	2	1	4
children's faeces							1[b]					1[b]	4[b]
grass											4		

[a] opinion split 50: 50 as to whether this is a source; [b] equal with livestock faeces; [c] equal with fish from river

Appendix 3.8 Rank of food sources of unowned dogs in order of importance (1 = most important).

	Antiga			Kelusa									
	Kaler (mixed)	Ketug (mixed)	Kelod (mixed)	Kelikikawan (mixed)	Roban (male)	Roban (female)	Ayah (male)	Ayah (female)	Peliatan (male)	Peliatan (female)	Triwangsa (male)	Yehtengah (male)	Yehtengah (female)
stealing leftovers from householders				6	1								
offerings	3	6	3	5	7	3	5	2	4	4	7	2	
rubbish	2		1	3	2		1	5		6	3	5	2
spoiled food in the ditch	4		5				4						1
school rubbish		3	4			2	7		7	5			
waste from the local stalls		5	2		5	1			6		1		3
waste food from someone other than the owner or neighbour	6	7	7						5				
pig food from the sty	1	2	8	9	3	4		3	2	3	5	3	6
stealing food from the local stalls				7			6						
stealing poultry				4	6		3						
dead animals	5	4	6	2				1	1	2	4	1	4
fish from the river				8									
vermin									3				
faeces		1		1	4								
livestock faeces							2	4		1	2	4	5
children's faeces							2[a]						5[a]
grass											6		
cassava in dry fields			9										

[a] equal with livestock faeces

Appendix 4

Appendix 4.1 Summary of the study methodology.

study area	location	cohort	vaccine used	vaccination strategy	blood sampling strategy	longitudinal data analysis
Zenzele	informal settlement in Gauteng Province, South Africa	research	Rabisin	door-to-door for 10 days every available dog in February 2010 not vaccinated by the DoA in October 2009	every available dog vaccinated in February 2010; samples collected day 0 (immediately prior to vaccination) and then 30, 90, 180 and 360 days after vaccination	all data summarised models were fitted with and without upper outliers[a]
		Department of Agriculture (DoA)	Rabisin Galaxy DA2PPv ivermectin	one day vaccination point (VP) October 2009	every available dog vaccinated in October 2009; samples collected 8-10, 30, 90, 180 and 360 days after vaccination	all data summarised titres excluded from models
Kelusa	inland village in Bali Province, Indonesia	research	Rabisin	door-to-door for 16 days every available dog in January 2010 not vaccinated by the DoL in December 2009	every available dog vaccinated and unvaccinated; samples collected 180 and 360 days after vaccination	all data summarised models were fitted to titres from vaccinated dogs models were fitted with and without upper outliers[b]
		Department of Livestock (DoL)	Rabivet Supra 92	one day vaccination point (VP) in two banjars in the study area December 2009		all data summarised titres excluded from models
Antiga	coastal village in Bali Province, Indonesia	research	Rabisin	door-to-door for 12 days every available dog in January 2010	every available dog vaccinated and unvaccinated; samples collected 180 and 360 days after vaccination	all data summarised models were fitted to titres from vaccinated dogs models were fitted with and without upper outliers[b]
		Department of Livestock (DoL)	Rabisin	one day vaccination point (VP) in one banjar outside the study area February 2010		all data summarised titres excluded from models

[a] upper outliers were those dogs in Zenzele (n=7) with histories and post-vaccinal titres suggestive of vaccination with Rabisin by the DoA through a vaccination point in Zenzele in May 2006 or undertaken independently by the owner

[b] upper outliers were those dogs in Kelusa and Antiga (n=4 and n=15 respectively) with histories and post-vaccination titres suggestive of vaccination undertaken independently by the owner or as part of vaccination campaigns outside of Kelusa and Antiga

Appendix 4.2 Summary of vaccination coverage.

	Zenzele research cohort	Zenzele DoA cohort	Kelusa research cohort	Antiga research cohort
method vaccine delivery	door-to-door	vaccination point (VP)	door-to-door	door-to-door
vaccination coverage day 0	82.2% (259/315)	26.9% (97/361)	80.9% (284/351)	79.2% (259/327)
	85.6% (332/388) incl. 73 dogs vaccinated by the DoA still present Feb-10[a]			
% sampled day 180[b]	_	_	75.2% (252/335)	76.8% (205/267)
% sampled & vaccinated day 360[b]	_	_	81.4% (250/307)	86.0% (202/235)
reasons for not vaccinating day 0	consent declined: 10 dogs	owner awareness of and access to vaccination point	consent declined: 3 dogs	consent declined: 7+ dogs[c]
	nobody home to obtain consent: 10 dogs		vaccinated by the DoL: 16 dogs[d]	could not be caught: 45 dogs
	unable to properly restrain: 11 dogs		could not be caught: 29 dogs	
	missed: 25 dogs		missed: 19 dogs	missed: 16 dogs
number consent declined or unable to restrain during the study period	consent declined for sampling: 3 dogs	consent declined for sampling: 13 dogs	consent declined for sampling: 14 dogs	consent declined for sampling: 13 dogs
	nobody home for consent: 21 dogs	nobody home for consent: 5 dogs	declined consent for vaccination day 360: 1 dog	declined consent for vaccination day 360: 1 dog
	unable to properly restrain: 12 dogs	unable to properly restrain: 7 dogs		

[a] there were an additional four households with at least one dog; three of these households owned mostly greyhounds that were confined to the yard
[b] in Kelusa 146 dogs and in Antiga 124 dogs were caught for blood sampling at both time points (including vaccinated and unvaccinated dogs)
[c] there was one household where the details of the number of dogs owned was not available
[d] see Appendix 4.24

Note: puppies born in the study area within ~12 weeks prior to vaccination/sampling but not observed during the vaccination/sampling period were assumed to have been lost prior to the commencement of vaccination/sampling

Appendix 4.3 The number of dogs in the research cohorts and the number of unvaccinated controls in Bali that were blood sampled at each time point.

	day 0[a]	day 30	day 90	day 180	day 360
Zenzele vaccinated dogs	190	183	148	134	103
Kelusa vaccinated dogs	–	–	–	168	124
Kelusa unvaccinated dogs	–	–	–	70	79
Antiga vaccinated dogs	–	–	–	163	126
Antiga unvaccinated dogs	–	–	–	35	49

[a] day 0 immediately prior to vaccination for the research cohort

Note 1: of the 97 dogs in Zenzele that were vaccinated by the DoA in October 2009, 60 were blood sampled 8-10 days after vaccination, and 77 were sampled 30 days, 65 were sampled 90 days, 64 were sampled 180 days, and 47 dogs were sampled 360 days after vaccination

Note 2: in addition to the dogs recorded in Appendix 4.3

- Kelusa day 180: 11 dogs vaccinated by the DoL Dec-09 sampled, 1 dog taken by the owner to an NGO for vaccination sampled, 1 dog sampled but its vaccination history was uncertain, 1 missing sample; there were an additional 32 puppies <6-8 weeks of age present in the study area during the sampling period
- Kelusa day 360: 12 dogs vaccinated by the DoL Dec-09 sampled, 1 dog taken by the owner to an NGO for vaccination sampled, 1 dog sampled but its vaccination history was uncertain, 33 dogs vaccinated only (mostly puppies <6-8 weeks of age)
- Antiga day 180: 3 dogs vaccinated by the DoL Apr-10 sampled, 1 dog taken by the owner to an NGO for vaccination sampled, 1 dog sampled but its vaccination history was unknown, 2 missing samples; there were an additional 19 puppies <6-8 weeks of age present in the study area during the sampling period
- Antiga day 360: 3 dogs vaccinated by the DoL Apr-10 sampled, 9 dogs in Ketug inadvertently vaccinated by the NGO involved in the island wide vaccination campaign sampled ~30 days after vaccinated (titres ranged from 2-181 IU/ml), 2 dogs taken by the owner to an NGO for vaccination sampled, 13 dogs vaccinated only (mostly puppies <6-8 weeks of age)

Appendix 4.4(i) Sample selection and sampling technique for deep skin scrapes.

Deep skin scrapes (DSS) were taken on day 180 in Bali to investigate generalised demodicosis. Having to catch affected dogs by net precluded true random selection, therefore a convenience sample of 15 dogs in Kelusa and 17 in Antiga were selected from the pool of 168 dogs diagnosed with generalised dermatitis during the preceding survey. To estimate the true prevalence of demodicosis in free-roaming dogs with dermatitis a sample size of approximately 90 dogs was required (AusVet EpiTools) [assuming an expected prevalence of 12% (Nayak *et al.* 1997; Rodriguez-Vivas *et al.* 2003), precision of 5%, and sensitivity of 90% and specificity of 99% based on operator experience and type of test (Fondati *et al.* 2009)]. Practical constraints limited testing to approximately 30 dogs with generalised dermatitis as a pilot study, considered sufficient to gauge the involvement of *Demodex spp.*

Lesions were arbitrarily classified as generalised according to the general literature on demodicosis (Mueller 2004; Miller, Griffin & Campbell 2013). Dogs with generalised dermatitis had 12 or more localised lesions, and/or contiguous lesions that may have included complete involvement of an entire body region (e.g. face, flank), and/or complete involvement of two or more feet.

Multiple deep skin scrapes, approximately 2x2cm, were taken from each affected dog according to standard protocols (Miller, Griffin & Campbell 2013). The cellular debris was placed into plain, coded 3ml blood tube and examined within 12 hours of sampling using a standard light microscope (from 4-40x magnification). Prior to microscopy, the samples were soaked in 10% potassium hydroxide for approximately 20 minutes to dissolve the cellular debris.

Appendix 4.4(ii) Sample selection for faecal analysis (see Appendix 4.25).

A prevalence of ~90% was expected for intestinal parasites in Zenzele, particularly *Anycylostoma spp.* (Minnaar, Krecke & Rajput 1999; Minnaar & Krecke 2001). In order to estimate true prevalence with a precision of 5% and test sensitivity and specificity of at least 95%, a sample of approximately 120 dogs was required. This estimate happened to coincide with the number of dogs (n=107) randomly selected to assess intestinal parasitism as a pilot study.

One dog was positive for *Hymenolepis nana*, a tapeworm found in humans and rodents, which was probably acquired from eating a rodent (Palmer *et al.* 2011).

Appendix 4.5 Age-specific life expectancies for the entire (ecological study) research population derived from the observed ages at the last time point of the study period (see Appendix 2.5).

current age (month of life)	age-specific life expectancy (years)		
	male	female	total
Zenzele			
2-12	2.8	3.0	2.9
13-24	3.1	2.3	2.7
25-36	3.5	4.1	3.7
37-48	4.8	3.6	4.3
49+			
Kelusa			
2-12	4.7	2.7	4.0
13-24	3.7	1.5	2.9
25-36	3.8	5.0	3.9
37+			
Antiga			
2-12	7.8	6.7	7.5
13-24	4.5	4.8	4.6
25-36	5.5	3.0	4.8
37+			

Appendix 4.6 Characteristics of the dogs in Bali (in January 2010) that were not vaccinated on day 0; the number that could not be caught for vaccination is shown in brackets.

age class[a] (month of life)	Kelusa[b]		Antiga[c]		
	male	female	male	female	
1-6	4 (0)	4 (0)	17 (6)	0 (0)	25
7-12	5 (2)	1 (0)	1 (1)	1 (1)	8
13-36	5 (4)	6 (5)	4 (4)	5 (4)	20
mature adult	18 (15)	5 (3)	26 (24)	7 (5)	56
	32	16	48	13	109

[a] dogs in age class 1-6 months are between birth [start of their 1st month of life] and ~26 weeks of age [end of their 6th month of life], and so on; mature adults are mostly dogs first observed as adults at the start of the (larger ecological) study period (i.e. from March 2008), but also dogs first observed as adults after the start of this study period, therefore the exact age of these dogs could not be determined by direct observation [Note: dogs in age classes 1-6, 7-12 and 13-36 months were observed as pups or juveniles from March 2008, so their true age was known]

[b] in addition, consent for vaccination was declined for 3 dogs including 1 male in its 20th month of life, 1 mature adult female, and 1 dog of unknown gender and age

[c] in addition, consent for vaccination was declined for at least 7 dogs including 5 mature adult males, 1 male in its 13th month of life, and 1 dog of unknown gender and age

Appendix 4.7 Description of the dogs in Zenzele with baseline (day 0) titres ≥0.5 IU/ml.

dog	day 0 IU/ml	day 30 IU/ml	present May 2006	gender	age (month of life)
1	1.00	45.25	yes	m	36+[a]
2	0.71	16.00	no	m	21
3	0.50	8.00	yes	m	36+
4	0.71	1.41	no	f	6
5	0.50	22.63	yes	m	36+

[a] ≥36th month of life in February 2010 (at vaccination)

Appendix 4.8 Summary of titres from the dogs in Zenzele necessary to assess the inadvertent inclusion of dogs vaccinated by the Department of Agriculture in October 2009 in the research cohort.

research cohort

day 0 IU/ml	frequency
0.06	79
0.09	40
0.13	15
0.18	25
0.25	16
0.35	10
0.50	2
0.71	2
1.00	1

research cohort upper outliers

dog	day 0 IU/ml	day 30 IU/ml
1	0.09	181.02
2	0.25	128.00
3	0.09	128.00
4	0.09	256.00
5	0.09	128.00
6	0.09	362.04
7	0.06	256.00

day 90 titres ≤ 1 IU/ml of dogs vaccinated by

dog	30 day IU/ml	90 day IU/ml
1	16.00	1.00
2	8.00	1.00
3	8.00	1.00
4	64.00	0.71
5	0.09	0.18
6	0.09	0.09
7	0.09	0.09
8	45.25	0.50
9	0.06	0.06
10	16.00	0.71
11	0.50	0.18
12	64.00	0.35
13	16.00	1.00

dogs vaccinated by the DoA

day 30 IU/ml	frequency
0.06	2
0.09	3
0.18	1
0.50	1
2.83	1
4.00	1
5.66	1
8.00	5
11.31	3
16.00	7
22.63	7
32.00	8
45.25	8
64.00	12
90.51	3
128.00	7
181.02	3
256.00	2
362.04	2

dogs vaccinated by the DoA with day 30 titres ≥128 IU/ml

dog	day 30 IU/ml	day 90 IU/ml
1	128.00	8.00
2	362.04	1.41
3	181.02	5.66
4	128.00	ns
5	362.04	11.31
6	128.00	5.66
7	128.00	8.00
8	181.02	22.63
9	256.00	128.00
10	128.00	ns
11	128.00	16.00
12	128.00	ns
13	256.00	2.83
14	181.02	32.00

ns = no sample

Appendix 4.9 Summary of the titres of the dogs in the Zenzele research cohort present May 2006 and those that arrived into the population after May 2006 (excluding 8 dogs whose presence in May 2006 was unknown; including upper outliers).

	day 0					day 30							day 360					
	observed GMT IU/ml	mode IU/ml	range IU/ml	no. with titres ≥ 0.5 IU/ml	Mann-Whitney test result	observed GMT IU/ml	mode IU/ml	range IU/ml	no. with titres < 0.5 IU/ml	no. with titres ≤ 0.1 IU/ml	no. upper outliers	Mann-Whitney test result	observed GMT IU/ml	mode IU/ml	range IU/ml	no. with titres < 0.5IU	no. with titres ≤ 0.1IU	Mann-Whitney test result
present May 2006	0.12	0.06	0.06 - 1.00	3 (5.1%)[a]	p=0.08	12.22	16.00 & 22.63	0.09 - 362.04	2 (3.7%)	1 (1.9%)	4	p=0.19	0.53	1.00	0.09 - 16.00	12 (33.3%)	2 (5.56%)	p=0.15
arrived after May 2006	0.10	0.06	0.06 - 0.71	2 (1.6%)[b]		16.38	22.63	0.09 - 256.00	3 (2.5%)	3 (2.5%)	1	(excluding upper outliers p=0.21)	0.77	1.00	0.06-5.66	26 (40.6%)	6 (9.4%)	

[a] 2 dogs with titres of 0.5 IU/ml and 1 dog with a titre of 1 IU/ml; [b] 2 dogs with titres of 0.71 IU/ml. The Mann-Whitney test was used to compare the mean titres between dogs present in Zenzele in May 2006 and those that arrived into the population after May 2006 (see Methods and materials section 4.2.4.2)

Appendix 4.10 Characteristics of the unvaccinated controls in Bali with titres ≥0.5IU/ml (n=15).

site and time point blood sampled	male	female
Kelusa day 180	4	3
Kelusa day 360	4	1
Antiga day 180	2[a]	0
Antiga day 360	2[a]	0

[a] one dogs was sampled at both time points

	age class (month of life)[a]			
site and time point blood sampled	1-6 months	7-12 months	13-36 months	mature adult
Kelusa day 180	4	1	0	2
Kelusa day 360	1	0	1	3
Antiga day 180	0	1	0	1[b]
Antiga day 360	0	0	1	1[b]

[a] see Appendix 4.6 for the definition; [b] one dog was sampled at both time points

titres IU/ml	**Kelusa**	**Antiga**
0.50	6	1[a]
0.71	3	1
1.00	1	2[a]
1.41	1	0
2.83	1	0

[a] one dog was sampled at both time points [day 180 titre = 1 IU/ml; day 360 titre = 0.5 IU/ml]

Appendix 4.11 Summary of the titres of the unvaccinated controls in Bali.

	day 180				day 360			
	observed GMT IU/ml	mode IU/ml	range IU/ml	Mann-Whitney test result	observed GMT IU/ml	mode IU/ml	range IU/ml	Mann-Whitney test result
Kelusa	0.10	0.06	0.04 - 1.41	p=0.13	0.09	0.06	0.04 - 2.83[a]	p<0.001 excluding Kelusa 2.83IU p<0.001
Antiga	0.09	0.04	0.04 - 1.00		0.06	0.04	0.04 - 0.71	

[a] 1 dog with a titre 2.83 IU/ml was acquired as a pup from outside the survey area (Ubud) and may have been vaccinated in Ubud; the Mann-Whitney test p-value remains <0.001 after removing this dog from the analysis. The Mann-Whitney test was used to compare the mean titres between unvaccinated dogs in Kelusa and Antiga for the same time points (see Methods and materials section 4.2.4.2)

Appendix 4.12 Characteristics (at vaccination) of the dogs in the Zenzele research and DoA cohort with peak (day 30) titres <0.5 IU/ml.

	id	day 30 IU/ml	gender	age (months)	present May-06	minimum body condition score	maximum body condition score	clinical signs	pregnancy or lactation
research cohort	1	0.09	m	8	no	4	4	yes	NA
	2	0.13	m	36+[a]	yes	me	me	no	NA
	3	0.09	f	15	no	2	3	me	lac
	4	0.09	f	21	no	2	5	yes	lac
	5	0.09	m	36+	yes	4	4	no	NA
	6	me[c]	m	36+	yes	me	me	me	NA
DoA cohort	1	0.09	m	32+[b]	yes	3	3	no	NA
	2	0.09	m	11	no	5	5	no	NA
	3	0.09	f	32+	yes	3	4	no	preg
	4	0.06	m	10	no	3	3	no	NA
	5	0.06	f	3	no	4	4	me	NA
	6	0.18	m	6	no	me	me	me	NA

[a] ≥36th month of life in February 2010 (at vaccination); [b] ≥32nd month of life in October 2009 (at vaccination)
[c] peak titre missing but titres <0.5IU/ml at all other time points; key: me = missing entry, NA = not applicable

Appendix 4.13 Summary of the day 180 and 360 titres in the research cohorts [vaccinated dogs] (excluding upper outliers).

	day 180						day 360					
	observed GMT IU/ml	mode IU/ml	range IU/ml	no. with titres < 0.5 IU/ml	no. with titres ≤ 0.1 IU/ml	Mann-Whitney test result	observed GMT IU/ml	mode IU/ml	range IU/ml	no. with titres < 0.5 IU/ml	no. with titres ≤ 0.1 IU/ml	Mann-Whitney test result
Zenzele	0.81	1.00	0.06 - 11.31	32 (24.8%)	4 (3.1%)	Zen: Kel p=0.015 Zen: Ant p=0.052	0.59	1.00	0.06 - 16.00	39 (39.4%)	8 (8.1%)	Zen: Kel p=0.025 Zen: Ant p=<0.001
Kelusa	1.08	0.71	0.04 - 8.00	37 (22.6%)	8 (4.9%)	Kel: Ant p=0.48	0.77	1.00	0.04 - 8.00	34 (27.4%)	8 (6.6%)	Kel: Ant p=0.11
Antiga	1.03	0.71	0.04 - 8.00	28 (18.9%)	4 (2.7%)	–	1.03	1.41[a]	0.04 - 11.31	25 (21.6%)	3 (2.6%)	–

[a] 16 dogs had titres of 1 IU/ml, 16 dogs had titres of 1.41 IU/ml, and 16 dogs had titres of 2 IU/ml. The Mann-Whitney test was used to compare the mean titres between vaccinated dogs in Zenzele, Kelusa and Antiga for the same time points (see Methods and materials section 4.2.4.2)

Appendix 4.14 Characteristics (at vaccination) of the dogs in the research cohorts with day 360 titres ≤0.1 IU/ml.

	dog	day 30 IU/ml	day 180 IU/ml	present May-06	gender	age (month of life)	minimum body condition score	maximum body condition score	clinical signs	generalised dermatitis	pregnancy or lactation
Zenzele research cohort (8.1%)	1	0.09	0.18	No	M	8	4	4	yes	NA	NA
	2	2.83	0.18	No	f	5	4	5	no	NA	NA
	3	5.66	0.06	No	m	2	me	me	me	NA	NA
	4	2.83	0.13	yes	m	36+[a]	4	5	yes	NA	NA
	5	4.00	me	No	f	7	me	me	me	NA	NA
	6	1.41	0.25	No	m	23	4	4	no	NA	NA
	7	0.09	0.13	no	f	21	2	5	yes	NA	lac
	8	0.09	0.09	yes	m	36+	4	4	no	NA	NA
Zenzele DoA cohort (6.4%)	1	2.83	0.18	no	f	32+[a]	me	me	me	NA	no
	2	me	me	no	m	32+	me	me	me	NA	NA
	3	0.06	0.25	no	f	3	4	4	me	NA	NA
Kelusa research cohort (6.6%)	1	NA	0.35	NA	m	1	me	me	me	me	NA
	2	NA	0.25	NA	m	34+[a]	4	4	yes	yes	NA
	3	NA	0.09	NA	m	3	me	me	me	me	NA
	4	NA	0.06	NA	f	10	me	me	me	me	no
	5	NA	0.18	NA	f	34+	me	me	me	me	no
	6	NA	0.06	NA	m	34+	5	5	yes	yes	NA
	7	NA	0.25	NA	m	9	4	4	me	me	NA
	8	NA	me	NA	m	25-33[b]	2	4	no	no	NA
Antiga research cohort (2.6%)	1	NA	me	NA	m	13-21[b]	me	me	me	me	NA
	2	NA	me	NA	m	33+[a]	me	me	me	me	NA
	3	NA	0.25	NA	m	33+	me	me	me	me	NA

[a] ≥36th month of life in February 2010 in Zenzele for the research cohort, 32nd month of life in Zenzele for the DoA cohort, 34th month in January 2010 in Kelusa, and 33rd month in January 2010 in Antiga (at vaccination); [b] month of life is in this range; key: me = missing entry, NA = not applicable

Appendix 4.15 Correlation coefficients for the dogs in the Zenzele research cohort that were blood sampled at every time point (i.e. day 30, 90, 180 and 360) (see Figure 4.1).

time points compared	correlation coefficient (*r*)			
	including dogs with day 30 titres <0.5 IU/ml and excluding upper outliers		including dogs with day 30 titres <0.5 IU/ml and including upper outliers	
	IU	log IU	IU	log IU
day 30 / 90	0.50	0.71	0.74	0.74
day 30 / 360	0.40	0.55	0.29	0.57
day 30 / mean 90-360	0.52	0.72	0.71	0.74
day 30 / mean 180-360	0.44	0.61	0.53	0.64
day 90 / 180	0.56	0.72	0.79	0.74
day 90 / 360	0.52	0.62	0.40	0.64
day 180 / 360	0.68	0.78	0.59	0.79

Appendix 4.16 Models restricted to natural log of the titre as the response variable and time as the covariate for the research cohorts. Natural logs are shown in the tables.

Model 1: **Zenzele** quadratic model fitted to four time points (day 30, 90, 180 and 360) with the intercept adjusted to day 30.

outliers	observations (n=)	AIC	parameter	estimate	2.5% CI	97.5% CI	p-value
included	568	1749.9	intercept	2.5917	2.4064	2.7769	<0.001
			time (Δ per day)	-0.0303	-0.0326	-0.0281	<0.001
			time^2 (Δ per day)	0.000064	0.000058	0.000071	<0.001
			std dev of dog-level random effect intercept	1.0029	0.8785	1.1449	–
excluded	547	1666.7	intercept	2.4957	2.3117	2.6796	<0.001
			time (Δ per day)	-0.0297	-0.0319	-0.0274	<0.001
			time^2 (Δ per day)	0.000063	0.000057	0.000070	<0.001
			std dev of dog-level random effect intercept	0.9661	0.8400	1.1112	–

Model 2: **Zenzele** linear model fitted to three time points (day 90, 180 and 360) with the intercept adjusted to day 90.

outliers	observations (n=)	AIC	parameter	estimate	2.5% CI	97.5% CI	p-value
included	385	1058.2	intercept	0.3016	0.1224	0.4807	0.001
			time (Δ per day)	-0.0034	-0.0040	-0.0027	<0.001
			std dev of dog-level random effect intercept	0.9747	0.8507	1.1167	–
excluded	371	1013.3	intercept	0.2542	0.0745	0.4339	0.006
			time (Δ per day)	-0.0033	-0.0039	-0.0026	<0.001
			std dev of dog-level random effect intercept	0.9588	0.8351	1.1009	–

Model 3: **Zenzele** linear model fitted to two time points (day 180 and 360) with the intercept adjusted to day 180.

outliers	observations (n=)	AIC	parameter	estimate	2.5% CI	97.5% CI	p-value
included	237	635.7	intercept	-0.1819	-0.3658	0.0021	0.054
			time (Δ per day)	-0.0018	-0.0026	-0.0010	<0.001
			std dev of dog-level random effect intercept	0.9611	0.8359	1.1050	–
excluded	228	610.2	intercept	-0.2120	-0.3979	-0.0261	0.026
			time (Δ per day)	-0.0018	-0.0026	-0.0010	<0.001
			std dev of dog-level random effect intercept	0.9493	0.8234	1.0945	–

Appendix 4.16 continued

Model 4: **Kelusa** linear model fitted to two time points (day 180 and 360) with the intercept adjusted to day 180.

outliers	observations (n=)	AIC	parameter	estimate	2.5% CI	97.5% CI	p-value
included	292	870.6	intercept	0.1464	-0.0443	0.3372	0.133
			time (Δ per day)	-0.0030	-0.0038	-0.0022	<0.001
			std dev of dog-level random effect intercept	1.1544	1.0212	1.3050	–
excluded	285	838.7	intercept	0.0918	-0.0960	0.2795	0.338
			time (Δ per day)	-0.0029	-0.0038	-0.0021	<0.001
			std dev of dog-level random effect intercept	1.1158	0.9839	1.2655	–

Model 5: **Antiga** linear model fitted to two time points (day 180 and 360) with the intercept adjusted to day 180.

outliers	observations (n=)	AIC	parameter	estimate	2.5% CI	97.5% CI	p-value
included	289	908.1	intercept	0.2687	0.0741	0.4634	0.007
			time (Δ per day)	-0.0012	-0.0022	-0.0001	0.030
			std dev of dog-level random effect intercept	1.0608	0.9176	1.2263	–
excluded	264	765.2	intercept	0.0208	-0.1568	0.1983	0.818
			time (Δ per day)	-0.0005	-0.0015	0.0005	0.356
			std dev of dog-level random effect intercept	0.8917	0.7579	1.0490	–

Note: in addition to excluding the 15 dogs with day 180 titres ≥11.3 IU/ml, excluding the 3 dogs with day 360 titres of 11.3 IU/ml results in observations = 258, intercept = 0.0907 p = 0.518, time = -0.0006 p = 0.213

Model 6: **Kelusa and Antiga** linear model fitted to two time points (day 180 and 360) with the intercept adjusted to day 180.

outliers	observations (n=)	AIC	parameter	estimate	2.5% CI	97.5% CI	p-value
included	581	1788.8	intercept	0.2071	0.0295	0.3847	0.023
			time (Δ per day)	-0.0021	-0.0027	-0.0014	<0.001
			std dev of site-level random effect intercept	0.0820	0.0091	0.7410	–
			std dev of site/dog-level random effect intercept	1.1060	1.0063	1.2157	–
excluded[a]	549	1616.6	intercept	0.0582	-0.0973	0.2168	0.378
			time (Δ per day)	-0.0017	-0.0024	-0.0010	<0.001
			std dev of site-level random effect intercept	0.00005	0.0000	0.2440	–
			std dev of site/dog-level random effect intercept	1.0054	0.9074	1.1106	–

[a] confidence intervals could not be obtained on the variance-covariance components using the intervals function with lme{nlme}; therefore, estimates of the confidence intervals were derived using the lme4 package with lmer, profile and confint functions

Appendix 4.17 Observed and predicted geometric mean titres for each time point. Predictions are derived from the models restricted to natural log of the titre as the response variable and time as the covariate (see Appendix 4.16).

		day 30 IU/ml	day 90 IU/ml	day 180 IU/ml	day 360 IU/ml
Zenzele	median (range) including upper outliers	22.63 (0.09 - 362.04)	1.41 (0.06 - 45.25)	1.00 (0.06 - 16.00)	0.71 (0.06 - 16.00)
	observed GMT including upper outliers	16.32	1.53	0.84	0.61
	median (range) excluding upper outliers	22.63 (0.09 - 90.51)	1.41 (0.06 - 16.00)	1.00 (0.06 - 11.31)	0.71 (0.06 - 16.00)
	observed GMT excluding upper outliers	14.80	1.45	0.81	0.59
	Appendix 4.16, model 1				
	predicted GMT (95CIs) including upper outliers	13.36 (11.09 - 16.07)	2.73 (2.31 - 3.24)	0.60 (0.49 - 0.74)	0.67 (0.54 - 0.85)
	predicted GMT (95CIs) excluding upper outliers	12.13 (10.09 - 14.58)	2.57 (2.17 - 3.03)	0.59 (0.48 - 0.71)	0.65 (0.52 - 0.82)
	Appendix 4.16, model 2				
	predicted GMT (95CIs) including upper outliers	–	1.35 (1.13 - 1.62)	1.00 (0.85 - 1.18)	0.54 (0.44 -0.66)
	predicted GMT (95CIs) excluding upper outliers	–	1.29 (1.08 - 1.54)	0.96 (0.81 - 1.13)	0.53 (0.43 - 0.65)
	Appendix 4.16, model 3				
	predicted GMT (95CIs) including upper outliers	–	–	0.83 (0.69 - 1.00)	0.60 (0.50 - 0.73)
	predicted GMT (95CIs) excluding upper outliers	–	–	0.81 (0.67 - 0.97)	0.59 (0.48 - 0.71)
Kelusa	median (range) including upper outliers	–	–	1.41 (0.04 - 16.00)	1.00 (0.04 - 11.31)
	observed GMT including upper outliers	–	–	1.16	0.80
	median (range) excluding upper outliers	–	–	1.41 (0.04 - 8.00)	1.00 (0.04 - 8.00)
	observed GMT exlcuding upper outliers	–	–	1.08	0.77
	Appendix 4.16, model 4				
	predicted GMT (95CIs) including upper outliers	–	–	1.16 (0.96 - 1.40)	0.67 (0.55 - 0.83)
	predicted GMT (95CIs) excluding upper outliers	–	–	1.10 (0.91 - 1.32)	0.64 (0.53 - 0.79)
Antiga	median (range) incl upper outliers	–	–	1.41 (0.04 - 45.25)	1.00 (0.04 - 16.00)
	observed GMT incl upper outliers	–	–	1.33	1.17
	median (range) excl upper outliers	–	–	1.00 (0.04 - 8.00)	1.00 (0.04 - 11.31)
	observed GMT excl upper outliers	–	–	1.03	1.03
	Appendix 4.16, model 5				
	predicted GMT (95CIs) incl upper outliers	–	–	1.31 (1.08 - 1.59)	1.06 (0.86 - 1.31)
	predicted GMT (95CIs) excl upper outliers	–	–	1.02 (0.85 - 1.22)	0.93 (0.77 - 1.14)

Appendix 4.18 Description of the covariates in the models described in Methods and materials section 4.2.4.2 (see Appendices 4.19-4.22).

The covariates were evaluated by direct observation, by the primary researcher and the enumerators, and by owner questionnaire. The questionnaire was developed and tested in February 2008 through community focus groups, including modified participatory rural appraisal (PRA) techniques (Chambers 1994a; Chambers 2007; Kumar 2007), and subsequent pilot studies. In Bali the questionnaires were bilingual (English and Bahasa with the Bahasa back-translated). Several languages were spoken in Johannesburg, including English, therefore the questions were written in English and the accuracy of the various translations checked regularly with the multi-lingual enumerators throughout the study period. The respondent was the person/s in the household that the householders collectively identified as most knowledge about the dog, which was not necessarily the owner. Respondents under 16 years of age were always interviewed with an adult present. Data collection was standardised through detailed enumerator training at the start of the study period for each enumerator and repeated on the first day of each survey. Surveys were undertaken every 6-12 weeks from March 2008 until April 2011. Each survey included the direct observations, by the primary investigator and enumerators, and the owner questionnaires. Apart from the primary researcher, all enumerators were local residents. All clinical examinations were undertaken by a qualified veterinarian.

The age of the dogs was reported by owners and/or visually assessed by the enumerators, including from the dentition in dogs in puppies and juveniles (i.e. dogs ≤12th month of life [≤ ~52 weeks of age] (Dyce, Sack & Wensing 1987) in Zenzele. The exact age (month of life) at vaccination was known for most dogs ≤ their 35th month in Zenzele, ≤33rd month in Kelusa, and ≤32nd month in Antiga (i.e. for dogs acquired from March 2008 as puppies or juveniles). Body condition includes the minimum and maximum score recorded during the surveys immediately prior to and following vaccination (i.e. within 6 weeks of vaccination). Body condition was evaluated using a standard 9-point (emaciated score 1 to obsess score 9) scoring system validated in adults (Laflamme 1997) and modified to assess body condition score (BCS) without palpation. The modified system was validated using dual energy x-ray absorptiometry in 71 dogs, including a small number of growing dogs (German & Holden 2006; German *et al.* 2006). Each dog was body condition scored independently by two enumerators during each survey. The minimum and maximum scores were generally the same or one score point different (e.g. 3 and 3, or 3 and 4 respectively). Clinical signs were

observed by the primary researcher and enumerators at the time of vaccination or during the survey immediately prior to vaccination. As part of the owner questionnaire, owners reported clinical signs they had observed during the previous 7 days and 3 months based on a set of pictures, each of a dog with a different clinical sign. With the exception of generalised dermatitis, clinical signs were those associated with serious illness and likely to cause weight loss, e.g. vomiting, lethargy. Almost all of the dogs with generalised dermatitis were in Bali, and were the majority of those with observed clinical signs. There was insufficient variation in reported protein intake in Zenzele for analysis. In Bali, protein intake (either never/rarely=0 or more frequent than never/rarely=1) reported during the survey immediately prior to vaccination was generally consistent with that fed throughout the study period.

Appendix 4.18 continued

covariate description, factor levels included in the analysis, and period of evaluation	methods of evaluation
house and dog identification	*What is the dog's name?* direct observation
gender (male or female)	direct observation
<u>at day 0 (vaccination):</u>	<u>at day 0 (vaccination):</u>
age class in months (month of life: 1-6, 7-12, 13-36, mature adult)[a]	*How old is this dog* or *When did you get this dog?* *How old was the dog when you got it?* direct observation
sterilization status (yes=1 / no=0)	*Has your (female) dog been sterilized* or *Has she had an operation to stop her having puppies?* direct observation of male dogs
pregnancy or lactation (yes=1 / no=0)	direct observation; whelping and vaccination dates
<u>Zenzele Jan-10 - Mar-10; Bali Dec-09 - Feb-10:</u>	
body condition minimum and maximum (thin [1-3] or fat [4+]; or 2, 3, 2-3, 4, 5, 5+ [also 6+ in Zenzele])	direct observation
<u>Zenzele Jan-10/Feb-10; Bali Dec-09/Jan-10:</u>	<u>Zenzele Jan-10/Feb-10; Bali Dec-09/Jan-10:</u>
clinical signs associated with serious illness[b] and generalised dermatitis (present=1 / not present=0)	direct observation and owner reporting based on a set of pictures In reference to the pictures:
generalised dermatitis as a separate category (present=1 / not present=0)	*Has this dog had any of <u>these problems</u> the <u>past 7 days</u>?* *Has this dog had any <u>other</u> problems the <u>past 7 days</u>?* *Has this dog had any of <u>these problems</u> since our <u>last visit</u>?* *Has this dog had any <u>other</u> problems since our <u>last visit?</u>*
<u>Bali Dec-09/Jan-10:</u>	<u>Bali Dec-09/Jan-10:</u>
protein intake (never or rarely=0 / more frequent than never or rarely=1)	owner reporting based on detailed discussions

[a] see Appendix 4.6 for the definition

[b] including, but not limited to, vomiting; diarrhoea; dysuria; dyschezia; constipation; swollen stomach; drooling/salivation; dehydration; increase or decrease in eating or drinking; recent weight loss; jaundiced, pale, hyperaemic or cyanotic mucous membranes; coughing; dyspnoea or tachypnoea; ataxia; lethargy/depression; recumbency; severe injury

Appendix 4.19 Zenzele models. The full range of models were tested with natural log of the titre as the response variable and the covariates described in Methods and materials section 4.2.3. All models with the lowest AIC retained time as the only covariate (i.e. for the quadratic models, see Appendix 4.16) or were null (i.e. for models fitted to a single time point) with the exception of the models shown below. Natural logs are shown in the tables.

Model 1: **Zenzele** quadratic model fitted to four time points (day 30, 90, 180 and 360) with the intercept adjusted to day 30. The model included natural log of the titre as the response variable and time, age, gender, pregnancy and lactation as the covariates. Only lactation at the time of vaccination was retained in the model with the lowest AIC.

outliers	observations (n=)	AIC	parameter	estimate	2.5% CI	97.5% CI	p-value
included	568	1745.9	intercept	2.6410	2.4534	2.8286	<0.001
			lactating (relative to not lactating)	-0.8562	-1.5414	-0.1710	0.015
			time (Δ per day)	-0.0303	-0.0325	-0.0281	<0.001
			time^2 (Δ per day)	0.000064	0.000058	0.000071	<0.001
			std dev of dog-level random effect intercept	0.9869	0.8618	1.1302	–
excluded	547	1663.3	intercept	2.5428	2.3563	2.7294	<0.001
			lactating (relative to not lactating)	-0.7886	-1.4541	-0.1232	0.021
			time (Δ per day)	-0.0297	-0.0319	-0.0274	<0.001
			time^2 (Δ per day)	0.000063	0.000056	0.000070	<0.001
			std dev of dog-level random effect intercept	0.9520	0.8293	1.0929	–

Model 2: **Zenzele** analysis of variance using stepAIC fitted to a single time point (day 30). The model included natural log of the titre as the response variable and age, gender, pregnancy and lactation as the covariates. Only lactation at the time of vaccination was retained in the model with the lowest AIC.

outliers	observations (n=)	AIC	parameter	estimate	2.5% CI	97.5% CI	p-value
included	183	135.8	intercept	2.8577	2.6408	3.0746	<0.001
			lactating (relative to not lactating)	-1.0902	-1.9748	-0.2056	0.016
excluded	176	116.4	intercept	2.7563	2.5436	2.9690	<0.001
			lactating (relative to not lactating)	-0.9888	-1.8395	-0.1381	0.023

Appendix 4.19 Zenzele models continued

Model 3: **Zenzele** analysis of variance using stepAIC fitted to a single time point (day 30). The model included natural log of the titre as the response variable and age, gender, pregnancy, lactation, clinical signs and body condition as the covariates. Only clinical signs at the time of vaccination was retained in the model with the lowest AIC. Clinical signs included generalised dermatitis because, in contrast to Bali, very few dogs in Zenzele had generalised dermatitis at the time of vaccination therefore this factor was not treated separately.

outliers	observations (n=)	AIC	parameter	estimate	2.5% CI	97.5% CI	p-value
included	115	89.6	intercept	3.0339	2.7034	3.3644	<0.001
			with clinical signs (relative to without clinical signs)	-0.6068	-1.1817	-0.0319	0.039
excluded	109	75.8	intercept	2.8509	2.5208	3.1810	<0.001
			with clinical signs (relative to without clinical signs)	-0.4239	-0.9830	0.1352	0.136

Model 4: **Zenzele** analysis of variance using stepAIC fitted to a single time point (day 0). The model included natural log of the lymphocyte counts as the response variable and age, gender, body condition and the natural log of day 30 titres as the covariates. Only body condition (in this case the lower of two independent scores) was retained in the model with the lowest AIC.

outliers	observations (n=)	AIC	parameter	estimate	2.5% CI	97.5% CI	p-value
NA	133	-186.64	intercept	1.276	1.1669	1.3860	<0.001
			body condition score 1-3 (relative to body condition 4+)	-0.189	-0.3607	-0.0170	0.032

Appendix 4.19 Zenzele models continued

Note 1: Models 1 and 2 treat the following dogs as not lactating at the time of vaccination: (i) three dogs that whelped in November 2009 and still had at least one of their pups present at vaccination (February 2010) but these pups were probably fully weaned (at vaccination), (ii) one dog that whelped in February 2010 but the survival/number of pups present at vaccination was uncertain, and (iii) two dogs that whelped in December 2009 and still had at least one of their pups present at vaccination but it was uncertain if the pups were fully weaned [treating these two dogs as lactating at vaccination in the quadratic model with upper outliers intercept = 2.6424, lactating = -0.7435 p = 0.022, and without upper outliers intercept = 2.5504, lactating = -0.8385 p = 0.010; and in the single time point model with upper outliers intercept = 2.8485, lactating = -0.7931 p = 0.059, and without upper outliers intercept = 2.7583, lactating = -0.9359 p = 0.025]

Note 2: One additional linear model, fitted to four time points (day 30, 90 180 and 360) with the intercept adjusted to day 30, with the lowest AIC retained covariates other than time. The model included natural log of the titre as the response variable and time, age, gender, pregnancy, lactation and body condition as covariates. The results were similar to Model 1 (i.e. the larger data set). Apart from time, the model with the lowest AIC retained lactation at the time of vaccination only. Treating the two dogs described under Note 1(iii) as not lactating at vaccination, with upper outliers observations = 449, intercept = 2.6664, lactating = -1.0848 p = 0.009, and without upper outliers observations = 432, intercept = 2.5599, lactating = -1.0097 p = 0.012. Treating the two dogs described under Note 1(iii) as lactating at vaccination, with upper outliers observations = 449, intercept = 2.6686, lactating = -0.8981 p = 0.016, and without upper outliers observations = 432, intercept = 2.5697, lactating = -1.0511 p = 0.006.

Note 3: One additional linear model, fitted to a single time point (day 30) with the lowest AIC, retained covariates. The model included natural log of the titre as the response variable and age, gender, pregnancy, lactation and body condition as covariates. The results were similar to Model 2 (i.e. the larger data set). The model with the lowest AIC retained lactation at the time of vaccination only. Treating the two dogs described under Note 1(iii) as not lactating at vaccination, with upper outliers observations = 144, intercept = 2.8825, lactating = -1.4486 p = 0.008, and without upper outliers observations = 138, intercept = 2.7756, lactating = -1.3417 p = 0.011. Treating the two dogs described under Note 1(iii) as lactating at vaccination, with upper outliers observations = 144, intercept = 2.8712, lactating = -0.9963 p = 0.043, and without upper outliers observations = 138, intercept = 2.7783, lactating = -1.2342 p = 0.013.

Note 4: One additional linear model, fitted to a single time point (day 30) with the lowest AIC, retained covariates. The model included natural log of the titre as the response variable and age, pregnancy, lactation and clinical signs as covariates. The results were similar to Model 3 (i.e. the smaller data set). The model with the lowest AIC retained clinical signs at the time of vaccination only. With upper outliers observations = 122, intercept = 2.9553, with clinical signs = -0.5365 **p = 0.063**. Including gender in the model, with upper outliers, treating the two dogs described under Note 1(iii) as lactating intercept = 2.9553, with clinical signs = -0.5365 **p = 0.063**; and, treating the two dogs described under Note 1(iii) as not lactating intercept = 3.2086, with clinical signs = -0.5583 p = 0.054, lactating = -0.8403 p = 0.14, male = -0.4230 p = 0.13. Without upper outliers all the models were null.

Appendix 4.20 Kelusa models. The full range of models were tested with natural log of the titre as the response variable and the covariates described in Methods and materials section 4.2.3. All models with the lowest AIC retained time as the only covariate (see Appendix 4.16) with the exception of the models shown below. Natural logs are shown in the tables.

Model 1: **Kelusa** linear model fitted to two time points (day 180 and 360) with the intercept adjusted to day 30. The model included natural log of the titre as the response variable and time, age, gender, protein intake, sterilization, clinical signs and generalised dermatitis as the covariates. Only generalised dermatitis at the time of vaccination was retained in the model with the lowest AIC. Clinical signs included dermatitis and in Kelusa the majority of dogs with clinical signs had generalised dermatitis

outliers	observations (n=)	AIC	parameter	estimate	2.5% CI	97.5% CI	p-value
excluded	205	590.8	intercept	0.8625	0.5362	1.1888	<0.001
			with generalised dermatitis (relative to without generalised dermatitis)	-0.3164	-0.7458	0.1130	0.150
			time (Δ per day)	-0.0034	-0.0043	-0.0024	<0.001
			std dev of dog-level random effect intercept	1.0696	0.9200	1.2435	–

Model 2: **Kelusa** linear model fitted to two time points (day 180 and 360) with the intercept adjusted to day 30. The model included natural log of the titre as the response variable and time, age, gender, protein intake, sterilization and body condition as the covariates. Only body condition at the time of vaccination was retained in the model with the lowest AIC. In this case body condition was the lower of the two independent scores.

outliers	observations (n=)	AIC	parameter	estimate	2.5% CI	97.5% CI	p-value
excluded	204	601.7	intercept	0.8966	0.5572	1.2359	<0.001
			body condition score 1-3 (relative to body condition 4+)	-0.3204	-0.7414	0.1007	0.138
			time (Δ per day)	-0.0035	-0.0045	-0.0025	<0.001
			std dev of dog-level random effect intercept	1.0693	0.9160	1.2483	–

Note 1: Two dogs with incomplete observational data for generalised dermatitis during December 2009 and January 2010 but with chronic, generalised dermatitis diagnosed by direct observation prior to December 2009 and after January 2010 almost certainly had generalised dermatitis when vaccinated. Model 1 treats these dogs as having generalised dermatitis at vaccination.

Note 2: One additional linear model, fitted to two points (day 180 and 360) with the intercept adjusted to day 30, with the lowest AIC retained covariates other than time. The model included natural log of the titre as the response variable and time, age, gender, protein intake, sterilization, body condition, clinical signs and generalised dermatitis as covariates. The results were similar to Model 1 (i.e. the larger data set). Apart from time, the model with the lowest AIC excluded upper outliers and retained generalised dermatitis at the time of vaccination only [observations = 183, intercept = 0.8961, with generalised dermatitis = -0.3358 p = 0.151].

Antiga 4.21 Antiga models. The full range of models were tested with natural log of the titre as the response variable and the covariates described in Methods and materials section 4.2.3. All models with the lowest AIC retained time as the only covariate (see Appendix 4.16) when upper outliers were included or were null when upper outliers were excluded with the exception of the models shown below. Natural logs are shown in the tables.

Model 1: **Antiga** linear model fitted to two time points (day 180 and 360) with the intercept adjusted to day 30. The model included natural log of the titre as the response variable and time, age, gender, pregnancy and lactation as the covariates. Only age class at the time of vaccination was retained in the model with the lowest AIC.

outliers	observations (n=)	AIC	parameter	estimate	2.5% CI	97.5% CI	p-value
included	289	905.6	intercept	0.8839	0.4012	1.3667	<0.001
			age class 7-12 months (relative to age class 1-6)	-0.7492	-1.4647	-0.0338	0.042
			age class 13-36 months (relative to age class 1-6)	-0.1143	-0.7661	0.5376	0.732
			age class mature adult (relative to age class 1-6)	-0.5920	-1.0751	-0.1089	0.018
			time (Δ per day)	-0.0011	-0.0022	-0.00008	0.037
			std dev of dog-level random effect intercept	1.0247	0.8831	1.1891	–

Model 2: **Antiga** linear model fitted to two time points (day 180 and 360) with the intercept adjusted to day 30. The model included natural log of the titre as the response variable and time, age, gender, pregnancy, lactation, clinical signs and generalised dermatitis as the covariates. Age class and generalised dermatitis at the time of vaccination were retained in the model with the lowest AIC. Clinical signs included generalised dermatitis and in Antiga the majority of dogs with clinical signs had generalised dermatitis.

outliers	observations (n=)	AIC	parameter	estimate	2.5% CI	97.5% CI	p-value
included	230	719.6	intercept	1.3406	0.7037	1.9774	<0.001
			with generalised dermatitis (relative to without generalised dermatitis)	-0.5383	-0.9292	-0.1475	0.008
			age class 7-12 months (relative to age class 1-6)	-0.9527	-1.7801	-0.1253	0.026
			age class 13-36 months (relative to age class 1-6)	-0.3015	-1.0850	0.4821	0.454
			age class mature adult (relative to age class 1-6)	-0.7956	-1.4182	-0.1729	0.014
			time (Δ per day)	-0.0011	-0.0023	0.00008	0.070
			std dev of dog-level random effect intercept	0.9872	0.8301	1.1741	–
excluded	208	596.0	intercept	0.1497	-0.0931	0.3925	0.227
			with generalised dermatitis (relative to without generalised dermatitis)	-0.3619	-0.7196	-0.0042	0.049
			std dev of dog-level random effect intercept	0.8648	0.7206	1.0380	–

Appendix 4.21 Antiga models continued

Model 3: **Antiga** linear model fitted to two time points (day 180 and 360) with the intercept adjusted to day 30. The model included natural log of the titre as the response variable and time, age, gender, pregnancy, lactation, body condition, clinical signs and generalised dermatitis as the covariates. Body condition and generalised dermatitis at the time of vaccination were retained in the model with the lowest AIC. In this case body condition was the higher of two independent scores. Clinical signs included generalised dermatitis and in Antiga the majority of dogs with clinical signs had generalised dermatitis.

outliers	observations (n=)	AIC	parameter	estimate	2.5% CI	97.5% CI	p-value
included	212	664.5	intercept	1.1138	0.5280	1.6996	<0.001
			with generalised dermatitis (relative to without generalised dermatitis)	-0.7244	-1.1520	-0.2968	0.001
			body condition score 5+ (relative to body condition score 2-3)	-0.5899	-1.1898	0.0099	0.057
			body condition score 4 (relative to body condition score 2-3)	-0.0352	-0.5792	0.5088	0.900
			time (Δ per day)	-0.0014	-0.0027	-0.0002	0.022
			std dev of dog-level random effect	1.0139	0.8528	1.2055	–
excluded	190	543.8	intercept	0.4577	-0.0322	0.9475	0.070
			with generalised dermatitis (relative to without generalised dermatitis)	-0.4893	-0.8742	-0.1044	0.014
			body condition score 5+ (relative to body condition score 2-3)	-0.5118	-1.0535	0.0299	0.067
			body condition score 4 (relative to body condition score 2-3)	-0.0653	-0.5578	0.4273	0.796
			std dev of dog-level random effect	0.8359	0.6855	1.0194	–

Note 1: Three dogs with incomplete observational data for generalised dermatitis during December 2009 and January 2010 but with chronic, generalised dermatitis diagnosed by direct observation prior to December 2009 and after January 2010 almost certainly had generalised dermatitis when vaccinated. Models 2 and 3 treat these dogs as having generalised dermatitis at vaccination.

Note 2: One additional linear models, fitted to two time points (day 180 and 360) with the intercept adjusted to day 30, with the lowest AIC retained covariates other than time. The model included natural log of the titre as the response variable and time, age, gender, pregnancy, lactation and body condition as covariates. The results were similar to Model 1 (i.e. the larger data set). Apart from time, the model with the lowest AIC included upper outliers and retained age class at the time of vaccination only [observations = 226, intercept = 1.0602, mature adults = -0.7044 p = 0.019, the other factor levels were non-significant].

Appendix 4.22 Bali models. The full range of models were tested with natural log of the titre as the response variable and the covariates described in Methods and materials section 4.2.3. All models with the lowest AIC retained time as the only covariate (see Appendix 4.16) with the exception of the models shown below. Natural logs are shown in the tables.

Model 1: **Bali villages** (Kelusa and Antiga) combined linear model fitted to two time points (day 180 and 360) with the intercept adjusted to day 30. The model included natural log of the titre as the response variable and time, age, gender, pregnancy and lactation as the covariates. Only lactation at the time of vaccination was retained in the model with the lowest AIC.

outliers	observations (n=)	AIC	parameter	estimate	2.5% CI	97.5% CI	p-value
included	581	1787.5	intercept	0.4944	0.2790	0.7097	<0.001
			lactating (relative to not lactating)	0.7373	-0.0569	1.5315	0.069
			time (Δ per day)	-0.0020	-0.0027	-0.00014	<0.001
			std dev of site-level random effect intercept	0.0629	0.0029	1.3483	–
			std dev of site/dog-level random effect intercept	1.1015	1.0017	1.2113	–

Model 2: **Bali villages** (Kelusa and Antiga) combined linear model fitted to two time points (day 180 and 360) with the intercept adjusted to day 30. The model included natural log of the titre as the response variable and time, age, gender, pregnancy, lactation, clinical signs and generalised dermatitis as the covariates. Lactation and generalised dermatitis at the time of vaccination were retained in the model with the lowest AIC. Clinical signs included generalised dermatitis and in Bali the majority of dogs with clinical signs had generalised dermatitis.

outliers	observations (n=)	AIC	parameter	estimate	2.5% CI	97.5% CI	p-value
included[a]	442	1353.7	intercept	0.7525	0.4987	1.0090	<0.001
			with generalised dermatitis (relative to without generalised dermatitis)	-0.3590	-0.6527	-0.0655	0.017
			lactating (relative to not lactating)	0.6723	-0.1960	1.5418	0.131
			time (Δ per day)	-0.0022	-0.0030	-0.0014	<0.001
			std dev of site-level random effect intercept	0.00005	0.0000	0.3021	–
			std dev of site/dog-level random effect intercept	1.0694	0.9537	1.1948	–
excluded	413	1199.4	intercept	0.5315	0.2870	0.7760	<0.001
			with generalised dermatitis (relative to without generalised dermatitis)	-0.3578	-0.6313	-0.0842	0.011
			time (Δ per day)	-0.0018	-0.0025	-0.0010	<0.001
			std dev of site-level random effect intercept	0.00004	0.0000[b]	0.2334[b]	–
			std dev of site/dog-level random effect intercept	0.9552	0.8461	1.0783	–

[a] confidence intervals could not be obtained on the variance-covariance components using the intervals function with lme{nlme}; therefore, estimates of the confidence intervals were derived using the lme4 package with lmer, profile and confint functions
[b] possible identifiability issues precluded estimates of the confidence intervals using the intervals function with lme{nlme}; rather estimates of the confidence intervals were derived using the lme4 package with the lmer, profile and confint functions

Appendix 4.22 Bali models continued

Note 1: Two dogs in Antiga had incomplete observational data for lactation at the time of vaccination but were almost certainly lactating when vaccinated (January 2010) based on the presence of puppies at vaccination and the bitches' whelping and vaccination dates. Models 1 and 2 treat these dogs as lactating at vaccination.

Note 2: Models 1 and 2 treat the following dogs as not lactating at the time of vaccination: (i) one dog in Antiga that did not whelp at the owner's house and, consequently, the survival/number of pups present at vaccination was uncertain, (ii) one dog in Kelusa that whelped in October 2009 and still had at least one pup present January 2010 but the pups were probably fully weaned at vaccination, and (iii) one dog in Kelusa that whelped in January but the vaccination date of the bitch was different to the puppies [treating this dog as lactating at vaccination in Model 1 (with upper outliers), given the likely scenario that she was vaccinated immediately after whelping and vaccination of the puppies was delayed until they were bigger, intercept = 0.4919, lactating = 0.7545 **p = 0.05**; treating this dog as lactating at vaccination in Model 2 (with upper outliers) intercept = 0.7484, lactating = 0.6726 p = 0.11, with generalised dermatitis = -0.3547 p = 0.019].

Note 3: One additional linear model, fitted to two time points (day 180 and 360) with the intercept adjusted to day 30, with the lowest AIC retained covariates other than time. The model included natural log of the titre as the response variable and time, age, gender, pregnancy and lactation (with the same inclusion/exclusion criteria for lactation as Models 1 and 2, see Notes 1 and 2 above). Treating the dog in Kelusa vaccinated ~ 2 weeks before her puppies (as per Note 2(iii)) as lactating at the time of vaccination, apart from time the model with the lowest AIC excluded upper outliers and retained lactation at vaccination only [observations = 549, intercept = 0.3020, lactating = 0.5822 p = 0.13].

Note 4: Two dogs in Kelusa (as per Table S21, Note 1) and three dogs in Antiga (as per Table S22, Note 1) with incomplete observational data for generalised dermatitis during December 2009 and January 2010 but diagnosed with chronic, generalised dermatitis by direct observation prior to December 2009 and after January 2010 almost certainly had generalised dermatitis when vaccinated. Model 2 treats these dogs as having generalised dermatitis at the time of vaccination.

Note 5: One additional linear model, fitted to two time points (day 180 and 360) with the intercept adjusted to day 30, with the lowest AIC retained covariates other than time. The model included natural log of the titre as the response variable and time, age, gender, pregnancy, lactation and body condition as covariates (with the same inclusion/exclusion criteria for lactation as Models 1 and 2, see Notes 1 and 2 above). The results were similar to Model 1 (i.e. the larger data set). Treating the dog in Kelusa vaccinated ~ 2 weeks before her puppies (as per Note 2(iii)) as not lactating at the time of vaccination, apart from time the model with the lowest AIC included upper outliers and retained lactation at vaccination only [observations = 437, intercept = 0.6646, lactating = 0.6837 p = 0.108]. Treating the dog in Kelusa vaccinated ~ 2 weeks before her puppies (as per Note 2(iii)) as lactating at vaccination, apart from time the model with the lowest AIC included upper outliers and retained lactation at vaccination only [observations = 437, intercept = 0.6617, lactating = 0.6959 p = 0.083].

Note 6: One additional linear model, fitted to two time points (day 180 and 360) with the intercept adjusted to day 30, with the lowest AIC retained covariates other than time. The model included natural log of the titre as the response variable and time, age, gender, pregnancy, lactation, body condition, clinical signs and generalised dermatitis as covariates (the same inclusion/exclusion criteria for lactation and generalised dermatitis as Models 1 and 2, see Notes 1, 2 and 4 above). The results were similar to Model 2 (i.e. the larger data set). With upper outliers and treating the dog in Kelusa vaccinated ~ 2 weeks before her puppies (as per Note 2(iii)) as not lactating at the time of vaccination, apart from time the model with the lowest AIC retained generalised dermatitis at the time of vaccination only [observations = 402, intercept = 0.8688, with generalised dermatitis = -0.3992 p = 0.012]; and, treating the dog in Kelusa vaccinated ~ 2 weeks before her puppies (as per Note 2(iii)) as lactating at vaccination, apart from time the model with the lowest AIC retained lactation and generalised dermatitis at vaccination [observations = 402, intercept = 0.8325, lactating = 0.6339 p = 0.137, with generalised dermatitis = -0.3705 p = 0.020]. Without upper outliers, apart from time the model with the lowest AIC retained generalised dermatitis at vaccination only [observations = 373, intercept = 0.5935, with generalised dermatitis = -0.3635 p= 0.014].

Appendix 4.23 Contingency tables of the covariates for the models described in Methods and materials section 4.2.4.2 (see Appendices 4.19-4.22). The tables show the maximum number of dogs with that factor level in the models that included upper outliers.

Zenzele

clinical signs		body condition		
yes	39	minimum	fat	19
			thin	20
		maximum	fat	24
			thin	15
no	78	minimum	fat	50
			thin	28
		maximum	fat	61
			thin	17

Kelusa

generalised dermatitis		body condition		
yes	44	minimum	fat	20
			thin	24
		maximum	fat	34
			thin	10
no	76	minimum	fat	51
			thin	25
		maximum	fat	66
			thin	10

Antiga

generalised dermatitis		body condition		
yes	60	minimum	fat	28
			thin	32
		maximum	fat	43
			thin	17
no	70	minimum	fat	49
			thin	21
		maximum	fat	60
			thin	10

Appendix 4.23 continued

Zenzele			
pregnancy		lactation	
yes	5	yes	11
no	187	no	181

Kelusa			
pregnancy		lactation	
yes	1	yes	2
no	182	no	181

Antiga			
pregnancy		lactation	
yes	2	yes	8
no	179	no	173

Kelusa	
protein	
yes	132
no	51

Antiga	
protein	
yes	139
no	40

Kelusa	
sterilised (males only)	
yes	34
no	149

Antiga	
sterilised (males only)	
yes	75
no	101

Appendix 4.24 Dogs vaccinated by the Department of Livestock in Kelusa with Rabivet Supra 92.

dog	days since vaccination	IU/ml	gender	age (month of life)
1	210	0.35	m	14
1	390	0.25		
2	210	0.25	m	33+[a]
2	390	0.50		
3	210	0.35	m	4
3	390	0.18		
4	210	0.06	m	33+
4	390	0.06		
5	210	0.35	f	5
5	390	0.71		
6	210	0.09	f	3
7	210	0.18	m	15
7	390	0.13		
8	390	0.25	f	8-16[b]
9	390	0.25	m	13
10	210	0.25	m	23
10	390	0.35		
11	210	0.50	m	4
11	390	0.35		
12	210	0.06	m	8
12	390	0.04		
13	390	0.25	m	14
14	210	0.35	f	2

[a] ≥33rd month of life in December 2009; [b] month of life in this range

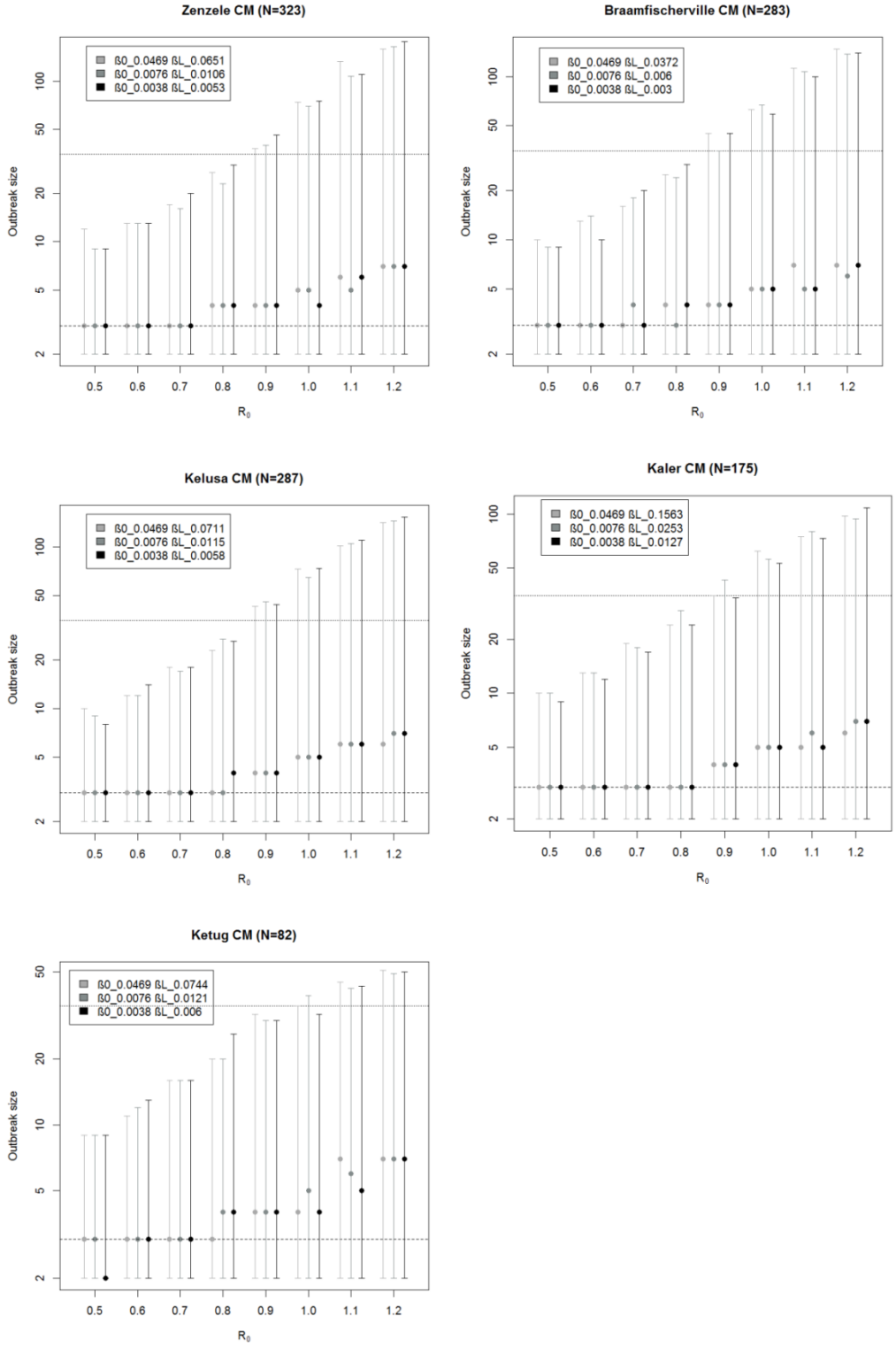
Note: this table excludes two mature adults (one male and one female) vaccinated by the Department of Livestock in December 2009 that died prior to the commencement of blood sampling in June 2010

Appendix 4.25 The number of dogs in Zenzele with intestinal parasites on day 0 (see Appendix 4.4.(ii)).

parasite	number of dogs diagnosed with the parasite
Ancylostoma	103
Coccidia	2
Spirocerca	6
Toxocara	5
Hymenolepis nana	1

Note: only 3 (of the 107 dogs tested) were negative for parasites

Appendix 5



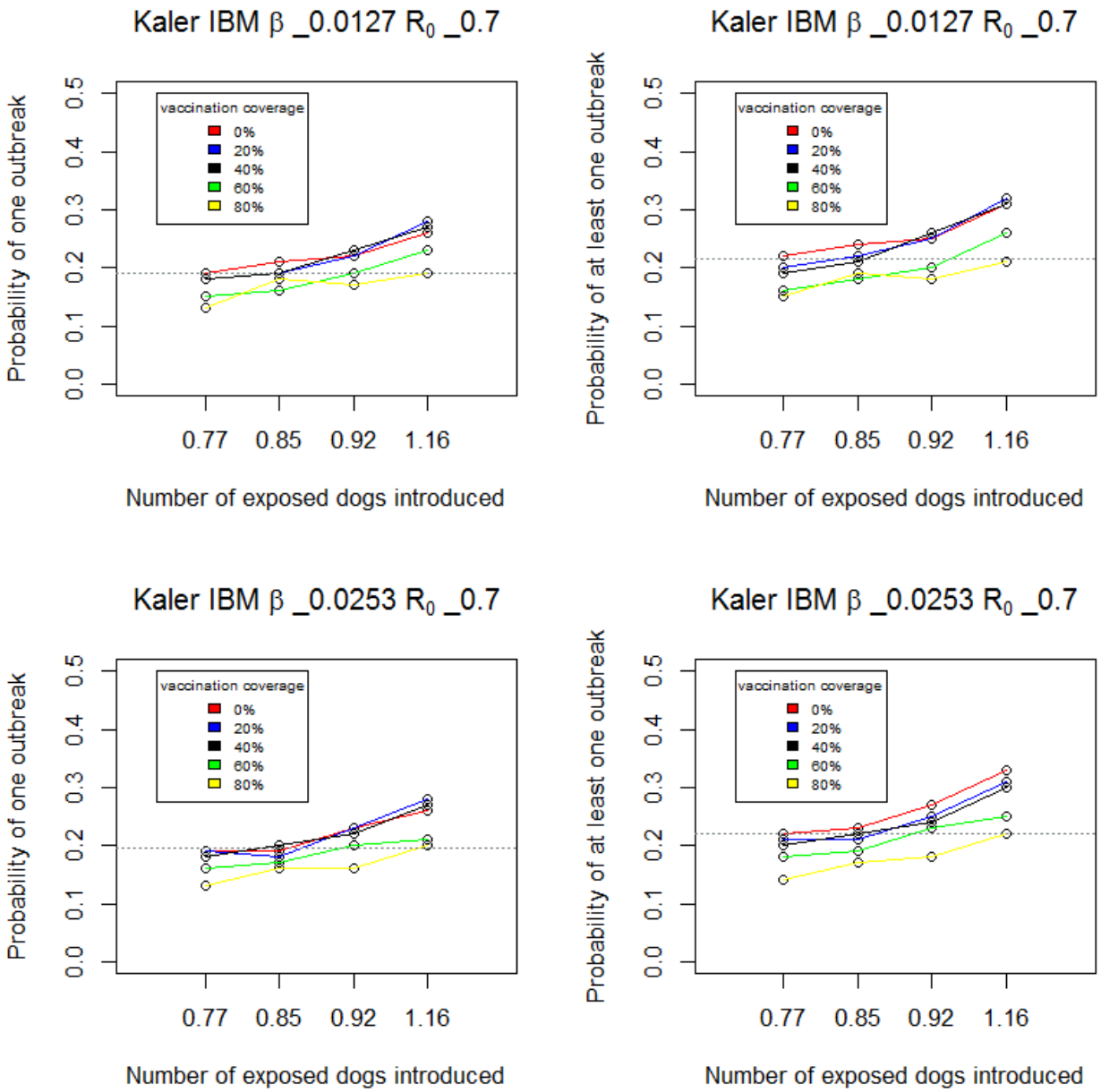
Appendix 5.1 Outbreak size for a range of R_0 and transmission rates (β) for the compartmental model (CM). Each vertical line represents the distribution in outbreak size generated from 1000 simulations. The dot on the vertical line represents the estimated median outbreak size. The top and bottom of the vertical line are equivalent to the estimated 95% and 5% quantile respectively. The top horizontal line indicates the upper limit in outbreak size (of approximately 35 cases) and the bottom horizontal line indicates the median outbreak size (of approximately 3 cases) observed in villages in the Serengeti District (Hampson *et al.* 2009). N = population size.

Appendix 5.2 Probability of an outbreak at the time of vaccination (t_0) and 12 and 24 months after vaccination for a range of vaccination coverage (from 0% to 80%) and transmission rates (beta).

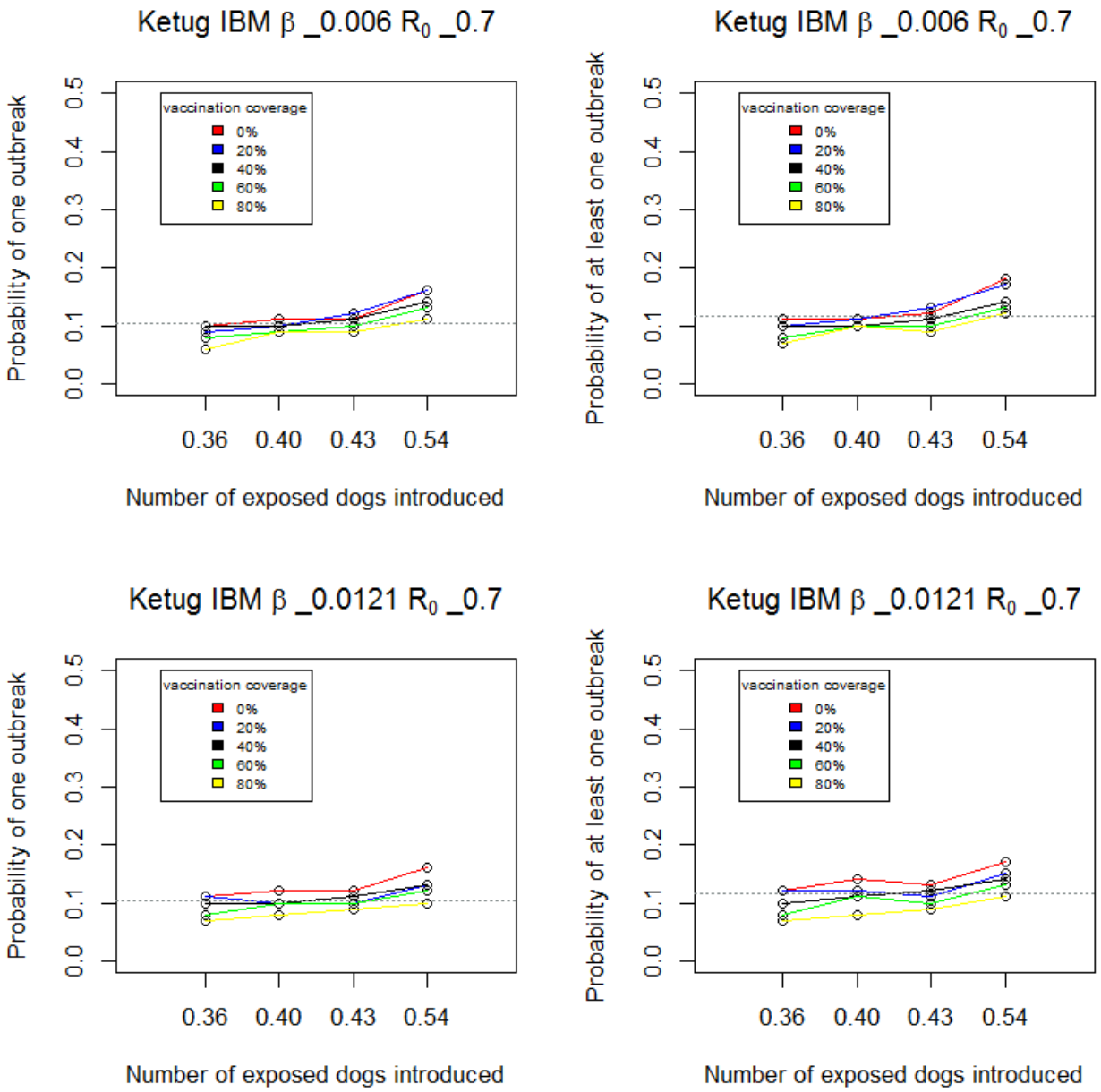
		Individual-based model						Compartmental model					
	vax cov	t_0	t_12	t_24	t_0	t_12	t_24	t_0	t_12	t_24	t_0	t_12	t_24
		Zenzele						Zenzele					
		beta_0.0651			beta_0.0053			beta_0.0651			beta_0.0053		
R_0_0.7	0	0.414	–	–	0.412	–	–	0.372	–	–	0.382	–	–
	20	0.373	0.364	0.364	0.368	0.359	0.358	0.325	0.348	0.369	0.320	0.360	0.370
	40	0.305	0.313	0.340	0.315	0.333	0.352	0.273	0.319	0.354	0.268	0.326	0.369
	60	0.217	0.274	0.295	0.227	0.275	0.331	0.208	0.309	0.323	0.192	0.306	0.337
	80	0.154	0.242	0.311	0.152	0.236	0.313	0.128	0.265	0.325	0.122	0.261	0.333
R_0_1.2	0	0.538	–	–	0.519	–	–	0.504	–	–	0.466	–	–
	20	0.463	0.496	0.482	0.501	0.481	0.462	0.472	0.496	0.497	0.431	0.485	0.476
	40	0.413	0.444	0.443	0.426	0.431	0.443	0.381	0.461	0.500	0.384	0.476	0.478
	60	0.351	0.407	0.430	0.345	0.409	0.410	0.275	0.427	0.479	0.322	0.427	0.459
	80	0.207	0.349	0.387	0.219	0.341	0.420	0.225	0.400	0.472	0.217	0.398	0.474
		Braamfischerville						Braamfischerville					
		beta_0.0372			beta_0.003			beta_0.0372			beta_0.003		
R_0_0.7	0	0.393	–	–	0.416	–	–	0.392	–	–	0.369	–	–
	20	0.341	0.338	0.361	0.336	0.345	0.344	0.330	0.348	0.369	0.307	0.356	0.376
	40	0.295	0.301	0.341	0.274	0.295	0.359	0.284	0.336	0.365	0.271	0.335	0.360
	60	0.221	0.272	0.337	0.213	0.286	0.340	0.218	0.296	0.354	0.229	0.314	0.347
	80	0.146	0.227	0.302	0.127	0.222	0.300	0.125	0.286	0.305	0.124	0.261	0.327
R_0_1.2	0	0.476	–	–	0.528	–	–	0.508	–	–	0.493	–	–
	20	0.451	0.443	0.485	0.461	0.443	0.527	0.464	0.521	0.509	0.431	0.472	0.488
	40	0.394	0.435	0.477	0.427	0.420	0.477	0.385	0.419	0.474	0.382	0.448	0.455
	60	0.313	0.381	0.466	0.326	0.340	0.458	0.276	0.399	0.468	0.287	0.410	0.483
	80	0.196	0.326	0.448	0.232	0.344	0.444	0.183	0.367	0.453	0.195	0.381	0.422
		Kelusa						Kelusa					
		beta_0.0711			beta_0.0058			beta_0.0711			beta_0.0058		
R_0_0.7	0	0.390	–	–	0.388	–	–	0.405	–	–	0.401	–	–
	20	0.344	0.367	0.400	0.336	0.353	0.399	0.343	0.373	0.394	0.335	0.367	0.366
	40	0.259	0.319	0.388	0.254	0.335	0.362	0.258	0.325	0.337	0.309	0.332	0.356
	60	0.208	0.268	0.321	0.214	0.295	0.347	0.232	0.296	0.341	0.209	0.299	0.321
	80	0.138	0.260	0.295	0.114	0.237	0.295	0.136	0.229	0.319	0.135	0.241	0.322
R_0_1.2	0	0.484	–	–	0.528	–	–	0.515	–	–	0.511	–	–
	20	0.458	0.463	0.546	0.476	0.515	0.521	0.474	0.480	0.482	0.479	0.474	0.484
	40	0.439	0.455	0.499	0.427	0.476	0.481	0.386	0.452	0.468	0.386	0.456	0.474
	60	0.288	0.410	0.458	0.321	0.414	0.469	0.313	0.439	0.475	0.314	0.401	0.444
	80	0.202	0.338	0.429	0.214	0.369	0.437	0.205	0.354	0.414	0.205	0.381	0.428
		Kaler						Kaler					
		beta_0.1563			beta_0.0127			beta_0.1563			beta_0.0127		
R_0_0.7	0	0.423	–	–	0.418	–	–	0.419	–	–	0.373	–	–
	20	0.330	0.358	0.371	0.308	0.325	0.373	0.340	0.328	0.389	0.297	0.373	0.384
	40	0.289	0.323	0.357	0.281	0.287	0.335	0.299	0.338	0.366	0.281	0.327	0.359
	60	0.197	0.280	0.303	0.220	0.286	0.319	0.216	0.293	0.355	0.207	0.285	0.354
	80	0.135	0.225	0.298	0.142	0.250	0.301	0.132	0.253	0.308	0.126	0.261	0.315
R_0_1.2	0	0.493	–	–	0.553	–	–	0.535	–	–	0.494	–	–
	20	0.490	0.475	0.489	0.438	0.445	0.537	0.453	0.492	0.465	0.479	0.489	0.505
	40	0.424	0.442	0.444	0.403	0.464	0.472	0.383	0.470	0.469	0.390	0.444	0.447
	60	0.329	0.402	0.447	0.330	0.381	0.452	0.326	0.431	0.476	0.318	0.386	0.431
	80	0.223	0.325	0.378	0.231	0.340	0.373	0.195	0.383	0.438	0.211	0.364	0.418
		Ketug						Ketug					
		beta_0.0744			beta_0.006			beta_0.0744			beta_0.006		
R_0_0.7	0	0.391	–	–	0.388	–	–	0.378	–	–	0.393	–	–
	20	0.366	0.353	0.381	0.360	0.345	0.394	0.327	0.369	0.371	0.337	0.352	0.355
	40	0.297	0.319	0.344	0.281	0.319	0.374	0.245	0.345	0.399	0.274	0.327	0.365
	60	0.214	0.281	0.333	0.221	0.282	0.340	0.197	0.295	0.321	0.225	0.309	0.315
	80	0.146	0.247	0.324	0.124	0.239	0.301	0.141	0.263	0.291	0.131	0.278	0.295
R_0_1.2	0	0.502	–	–	0.524	–	–	0.512	–	–	0.517	–	–
	20	0.481	0.488	0.544	0.492	0.489	0.519	0.428	0.479	0.473	0.467	0.480	0.480
	40	0.428	0.457	0.495	0.425	0.454	0.508	0.401	0.450	0.488	0.382	0.432	0.471
	60	0.301	0.405	0.477	0.327	0.395	0.471	0.321	0.414	0.455	0.286	0.409	0.466
	80	0.214	0.379	0.413	0.224	0.356	0.425	0.194	0.344	0.416	0.172	0.354	0.427

Appendix 5.3 Mean estimates of vaccination coverage with time (95% confidence intervals).

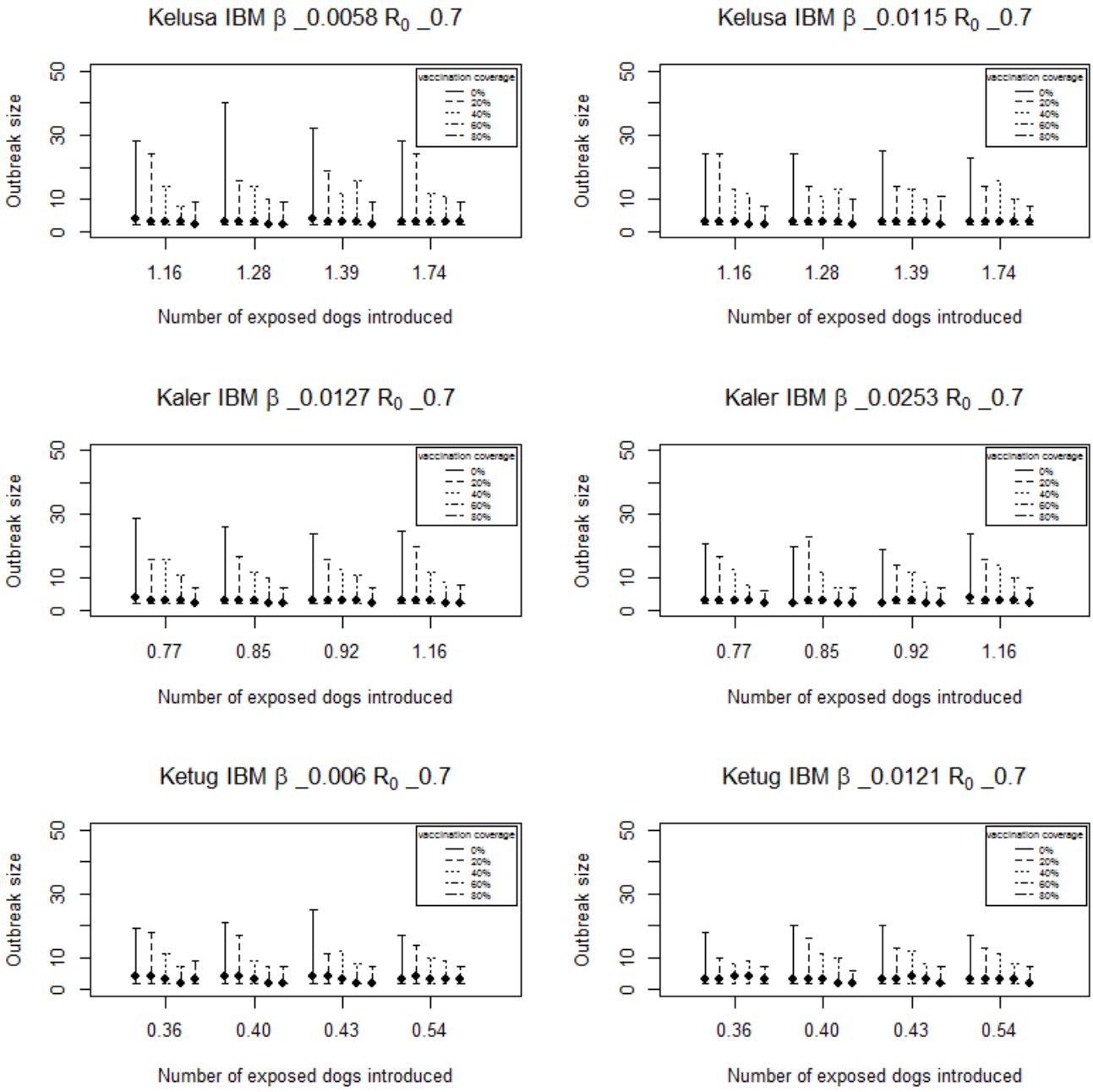
	% of the population vaccinated at month 0	% vaccination coverage after month 12	% vaccination coverage after month 24
Zenzele	60	39.4 (39.3-39.5)	25.1 (25.0-25.2)
	80	52.6 (52.5-52.6)	33.5 (33.5-33.6)
Braamfischerville	60	38.3 (38.2-38.4)	24.0 (23.9-24.1)
	80	50.9 (50.8-51.0)	29.3 (29.2-29.3)
Kelusa	60	37.8 (37.7-37.8)	26.3 (26.3-26.4)
	80	50.5 (50.4-50.6)	35.2 (35.1-35.3)
Kaler	60	42.5 (42.4-42.5)	32.6 (32.4-32.7)
	80	56.4 (56.4-56.5)	43.4 (43.2-43.4)
Ketug	60	39.8 (39.7-40.0)	26.8 (26.6-26.9)
	80	52.9 (52.7-53.0)	35.4 (35.3-35.5)



Appendix 5.4 Probability of outbreaks with human-mediated incursions for a range of vaccination coverage at the start of the time series and transmission rates (β) for Kaler. The plots were generated using the individual-based model (IBM). The probability of an outbreak during the 37 month study period is shown on the y-axis. The average number of exposed dogs entering the study population during the study period are shown on the x-axis; and were estimated from empirical data described in section 5.2.3.3. The baseline probability that a dog gained into the study population during the study period was exposed was obtained by dividing 0.77 (number of exposed dogs) by the total number of dogs gained into the study population during the study period (n=266). This probability was included in the model. The number (and the probability) of exposed dogs gained during the study period was then increased incrementally by 10%, 20% and 50% from the baseline as shown. The horizontal line provides a reference to allow for comparisons.



Appendix 5.5 Probability of outbreaks with human-mediated incursions for a range of vaccination coverage at the start of the time series and transmission rates (β) for Ketug. The plots were generated using the individual-based model (IBM). The probability of an outbreak during the 37 month study period is shown on the y-axis. The average number of exposed dogs entering the study population during the study period are shown on the x-axis; and were estimated from empirical data described in section 5.2.3.3. The baseline probability that a dog gained into the study population during the study period was exposed was obtained by dividing 0.36 (number of exposed dogs) by the total number of dogs gained into the study population during the study period (n=116). This probability was included in the model. The number (and the probability) of exposed dogs gained during the study period was then increased incrementally by 10%, 20% and 50% from the baseline as shown. The horizontal line provides a reference to allow for comparisons.



Appendix 5.6 Outbreak size with human-mediated incursions for a range of vaccination coverage at the start of the time series and transmission rates (β) for all of the Bali study populations. The plots were generated using the individual-based model (IBM). The number of exposed dogs gained into the study population during the study period are shown on the x-axis; see the legends to Figure 5.3 and Appendices 5.4 and 5.5 for the derivation of the number (and probability) of exposed dogs gained into the study population during the study period. Each vertical line represents the distribution in outbreak size generated from 1000 simulations. The dot on the vertical line represents the estimated median outbreak size. The top and bottom of the vertical line are equivalent to the estimated 95% and 5% quantile respectively.

Appendix 6

Appendix 6.1 Rabies vaccine-induced virus neutralizing antibody (VNA) titres in pups in Zenzele.

Excerpt from *M.K Morters, S McNabb, D.L Horton, A.R Fooks, J.P Schoeman, H.R Whay, J.L.N Wood, S Cleaveland (in preparation) Effective vaccination against rabies in puppies in rabies endemic regions*

Every available puppy (≤12 weeks of age) in Zenzele was vaccinated in February 2010 as part of the serological study described in Chapter 4 [68 puppies were vaccinated from a total of 86 in the population]. The majority of the puppies were <6-8 weeks of age when vaccinated and deemed too small to blood sample immediately prior to vaccination without causing unnecessary distress to the puppy and/or owner. Therefore, pre-vaccinal virus neutralizing antibody titres were obtained from only four of the puppies. Titres for these puppies were ≤0.13 IU/ml, similar to pre-vaccinal titres in 32 dogs 1.5-4.5 months of age from dams vaccinated with high quality, inactivated vaccine against rabies in Thailand (Kasempimolporn *et al.* 1996). To measure post-vaccinal peak titres blood samples were collected approximately 30 days following vaccination. Thirty seven of the 68 vaccinated puppies remained in Zenzele 30 days after vaccination, and of these nineteen were big enough to blood sample; peak titres for these (19) puppies are shown in the table below. All of the puppies seroconverted to the vaccine (i.e. generated titres ≥0.5 IU/ml (Kennedy 1998)), with a geometric mean titre (GMT) of 20.7 IU/ml.

Appendix 6.1 continued

Day 0 (pre-vaccination) and day 30 (peak) titres of the puppies vaccinated in Zenzele

dog	gender	age at vaccination (weeks)	puppy day 0 titres (IU/ml)	puppy day 30 titres (IU/ml)	dam day 0 titres (IU/ml)	dam present May 2006
1	f	8-10	0.06	11.3	0.06	yes
2	m	8-10	0.06	2.0	0.06	yes
3	f	7-8	–	45.3	0.18	yes
4	m	6-7	–	22.6	0.06	no
5	m	6-7	–	45.3	0.06	no
6	f	7-8	–	16.0	0.06	no
7	m	5-6	–	64.0	0.06	no
8	f	4-6	–	45.3	0.06	yes
9	f	5-7	–	32.0	0.06	yes
10	f	5-7	–	64.0	0.06	yes
11	m	5-7	–	5.7	0.06	yes
12	m	4-6	–	45.3	0.18	yes
13	f	4-6	–	90.5	0.18	yes
14	f	5	–	22.6	0.06	yes
15	f	5	–	8.0	0.06	yes
16	f	5	–	5.7	0.06	yes
17	f	10 days	–	5.7	0.09	no
18	f	6-8	–	32.0	–	–
19	f	10-12	–	22.6	–	–

Seventeen of the nineteen puppies (blood sampled on day 30) were born in Zenzele to eight dams; all of the adult females were seronegative (<0.5 IU/ml) immediately prior to vaccination in February 2010 (titres ranged from 0.06-0.18 IU/ml). Five of the dams may have been vaccinated by the Department of Agriculture in May 2006, however none had an anamnestic response to vaccination (see Chapter 4, section 4.3.3) in February 2010 (day 30 titres ranged from 0.09-90.5 IU/ml). The other two puppies (blood sampled on day 30) were obtained from outside Zenzele, therefore the vaccination status of their dams was not known. Only five of the (68) vaccinated puppies were still in Zenzele 90 days after vaccination, and of these four remained 12 months after vaccination (with day 360 titres of 0.09, 0.35, 0.35 and 1 IU/ml).

Appendix 7

Appendix 7.1 Transcription errors.

Paper questionnaires were used to collect dog and household data in the study areas (see Chapter 2, section 2.2.2 and Appendix 2.1). Each questionnaire included up to 100 questions, with the number of questioned asked during the interview dependent on the gender of the dog and the acquisition and loss of dogs from the household since the previous survey. In order to estimate the number of transcription errors incurred during the transfer of data from the questionnaires to the Excel spreadsheet (used to store and manipulate the data) approximately 400 questionnaires were randomly selected from all of the questionnaires completed during the study period (March 2008 – April 2011). A sample size of 400 gives 95% power to detect errors occurring at 1% frequency (Thrusfield 2005). The data in each sample questionnaire was checked against the corresponding data in the spreadsheet. An error rate of approximately 0.5% was detected.

Summary of the transcription errors

	Zenzele	**Braamfischerville**	**Kelusa**	**Antiga**	total
number of questionnaires selected	132	90	137	113	472
number of entries checked	8064	5202	8649	6889	28804
number of errors	41	18	42	49	150
% of entries with errors	0.51	0.35	0.49	0.71	**0.52**
number of questionnaires with errors	24	14	26	22	86
% of questionnaires with errors	18.18	15.56	18.98	19.47	18.22

Appendix 7.2 Repeatability of the questionnaires.

Repeatability of the questionnaire was tested in Braamfischerville in September 2010 and in Antiga in April 2010 by re-interviewing the owners of 34 randomly selected dogs in each site. The first and second interviews were one day apart. The same questionnaire was used and the same respondent interviewed. The respondents had no prior knowledge of the repeat interview. The enumerator undertaking the repeat interview was randomly allocated. A comparison of the responses given during the first and second interview are shown in the table below. Comparisons exclude questions and observations specific to the previous 7 days. For example, questions such as *Have you taken this dog out of the village the past 7 days?* and *When did you last feed this dog?* and the clinical signs observed by the primary researcher and enumerator during the first interview were excluded from the analysis. This was on the basis that it would not be possible to differentiate any difference in the response due to an actual change in circumstance from variability in owner recall.

Comparison of the responses given during the first and second interviews

		Braamfischerville	**Antiga**
current age (1-6, 7-12, 13-36, 37+ months)	same category	27 (79.4%)	21 (84.0%)
	one category different[a]	7 (20.6%)	4 (16.0%)
age at acquisition (≤3[b], 4-12, 13+[c] months)	same category	34 (100%)	31 (91.2%)
	different category	0 (0%)	3 (8.8%)
source of a new dog[d]	same location	6 (100%)	3 (100%)
	different location	0 (0%)	0 (0%)
body condition score (1-9) (primary researcher)	same score	30 (88.2%)	12 (54.5%)
	one score different	4 (11.8%)	10 (45.5%)
owner reported clinical signs previous 3 months	same	30 (85.3%)	31 (91.2%)
	different	4 (14.7%)	3 (8.8%)

[a] there was ≤ 6 month difference in reported age for 6 dogs and >6 month difference in reported age for 5 dogs; [b] understood by the enumerators to be ≤12 weeks of age; [c] understood by the enumerators to be older than 1 year (52 weeks) of age
[d] there were only 6 and 3 new dogs present in the selected households in Braamfischerville and Antiga respectively

Note: In Antiga n=9 respondents reported 'don't know' regarding the current age of the dog during one or both of the interviews. In Antiga n=12 dogs were not present during one or both of the interviews, therefore these dogs could not be given a body condition score; the variability in body condition scores in Antiga is probably the result of increased flightiness and movement of the dogs as a consequence most of the dogs having been recently caught by net for vaccination.